ABSTRACT

Title of Dissertation: INFLUENCE OF ACUTE AND CHRONIC EXERCISE ON MARKERS OF HIPPOCAMPAL PLASTICITY

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Exercise and physical activity are lifestyle behaviors associated with enriched mental health. Understanding the mechanisms by which exercise and physical activity improve mental health may provide insight for novel therapeutic approaches for numerous mental health disorders. This dissertation reports the findings from three studies investigating the influence of acute and chronic exercise on behavioral and mechanistic markers of hippocampal plasticity and delves into the potential role of noradrenergic signaling in the hippocampal adaptations with exercise. The first study assessed the effects of long-term voluntary wheel running on hippocampal expression of plasticity-associated genes and proteins in adult male and female C57BL/6J mice, highlighting sex differences in the adaptations to long-term voluntary wheel running. The second study examined the influence of acute exercise intensity on AMPA receptor phosphorylation, a mechanism essential for hippocampal plasticity, plasticity-associated gene expression, spatial learning and memory, and anxiety-like behavior. The unexpected finding that acute exercise increased anxiety-like

behavior encouraged investigation into the role of central noradrenergic signaling in acute exercise-induced anxiety. The third study determined how previous exposure to voluntary wheel running modulates the response to an acute bout of exercise, focusing primarily on transcription of the important plasticity-promoting gene, brain-derived neurotrophic factor. Using a pharmacological approach to compromise the locus coeruleus noradrenergic system, a system that is implicated in age-related mental health disorders such as Alzheimer's Disease, the third study also investigated the influence and interaction of the noradrenergic system and acute exercise on expression of multiple brain-derived neurotrophic factor transcripts. Together, this dissertation reports the findings from a series of experiments that explored similarities, differences, and interactions between the effects of acute and chronic exercise on markers of hippocampal plasticity and behavior. Further, this work provides insight into the role of the noradrenergic system in exercise-induced hippocampal plasticity.

Influence of Acute and Chronic Exercise on Markers of Hippocampal Plasticity

by

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List of Abbreviations

β_2AR*:* β_2-adrenergic receptor; official gene symbol: *Adrb2*
ActB: Actin, beta
ACTH: Adrenocorticotropin Hormone
AMPA: α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
AMPAR: AMPA receptor
AUC: Area under the curve
BCA: Bicinchoninic acid
BDNF: Brain-derived neurotrophic factor
BDNF: Protein in humans *
BDNF: Gene in humans*
Bdnf: Protein in rodents*
Bdnf: Gene or mRNA in rodents*
CAMKII: Calcium/calmodulin-dependent protein kinase II
CNS: Central nervous system
CREB: cAMP response element binding protein
DSP-4: N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride
ERK: Extracellular regulated MAP kinase
fMRI: Functional magnetic resonance imaging
Gapdh: Glyceraldehyde-3-phosphate dehydrogenase
GluR1: Glutamate ionotropic receptor AMPA type subunit 1; Official gene symbol: *Gria1*
HPA: Hypothalamus-pituitary-adrenal
IGF-1: Insulin like growth factor 1
IP: Intraperitoneal
IPGTT: Intraperitoneal glucose tolerance testing
KO: Genetic knockout
LC: Locus coeruleus
LTP: Long-term potentiation
mGluR: Metabotropic glutamate receptor
MHPG: 3-methoxy-4-hydroxyphenylglycol
mRNA: Messenger ribonucleic acid
NA: Noradrenergic
NMDA: *N*-methyl-d-aspartate
NMDAR: NMDA receptor
NOP: Novel object placement
NR2A: glutamate receptor, ionotropic, NMDA2A; official gene symbol: *Grin2a*
NR2B: Glutamate receptor, ionotropic, NMDA2B; official gene symbol: *Grin2b*
NT4: Neurotrophin 4
NTS: Nucleus of the solitary tract
PGC-1a: Peroxisome proliferator-activated receptor γ coactivator 1 alpha; official gene symbol: *Ppargc1a*
PKA: Protein kinase A
PTSD: Post-traumatic stress disorder

PVN: Paraventricular nucleus of the hypothalamus
qPCR: Quantitative polymerase chain reaction
SDS: Sodium dodecyl sulfate
Ser831: Serine 831
Ser845: Serine 845
tPa: Tissue plasminogen activator; official gene symbol: *Plat*
TrkB: Neurotrophic receptor tyrosine kinase 2; official gene symbol: *NTRK2*
UMD: University of Maryland
VO_2max: Maximal oxygen consumption

*This is the format for all official gene and protein names in the document. Capitalization may vary when common abbreviations are used instead of official gene symbols (e.g. GluR1, NR2A, NR2B, TrkB). Genes and mRNAs are italicized throughout the document.

Chapter 1. Introduction and Specific Aims

Physical activity promotes brain health by inducing adaptations that increase hippocampal volume, improve hippocampal-dependent learning and memory, decrease anxiety and depression, and lower the risk of cognitive impairment and dementia later in life. The overall aim of this dissertation project is to identify potential mechanisms mediating these favorable adaptions to exercise. The literature reporting the cognitive benefits of exercise training and chronic physical activity in humans and rodents is vast. Non-human studies examining the mechanisms of plasticity induced by chronic exercise have primarily focused on "short-term" chronic exercise exposures (< one month) that may reflect adaptations in response to the novelty of the activity. In rodents, voluntary running activity decreases over time, so utilizing short-term exercise studies may favor plasticity by capturing the novelty of running and high wheel activity. Further, studies typically use only one sex in the sample population, which has limited our understanding of any potential sex differences in the response to exercise training. Chapter Three of this dissertation contains a manuscript published in *Physiology and Behavior* in 2015 that reports a series of experiments that tested the hypotheses of Specific Aim 1.

Aim #1: Determine the effect of five months of voluntary wheel exposure on hippocampal mRNA expression of plasticity-associated genes in adult male and female mice.

Hypothesis #1: Due to the gradual reduction in voluntary wheel activity over time, plasticity-associated gene expression will not differ between exercise and sedentary mice.

Exploratory Hypothesis #1: Any observed differences in mRNA expression between exercise and sedentary mice will be sex-dependent.

The purpose of this investigation was to determine the influence of long-term (20 weeks) voluntary wheel running on expression of genes known to play critical roles in exercise-induced enhancement of brain health in rodents. Moreover, our sample consisted of both male and female C57BL/6J mice, providing the opportunity to investigate sex differences in the response to long-term voluntary wheel running. We observed that long-term wheel running increased the expression of a critical neurotrophin for brain health and plasticity, brain-derived neurotrophic factor (*Bdnf*), in a transcript and sex specific pattern. Bdnf protein analysis concurred with the mRNA data, demonstrating that long-term voluntary wheel running increases Bdnf mRNA and mature protein only in male mice.

In contrast to the large number of studies examining the mechanisms of chronic exercise training, research examining the effects of acute bouts of exercise on brain health has been limited to correlational investigations of peripheral markers in human participants or investigations in rodents using multiple-day “acute” exercise interventions instead of true single acute bouts of exercise. The mechanisms mediating the effects of chronic exercise training are likely due to accumulated changes induced by repeated bouts of acute exercise,

and characterizing the immediate benefits of acute exercise is essential to understanding and optimizing exercise training and future "exercise-like" interventions for hippocampal plasticity. Bdnf is known to be essential for exercise-induced improvements in memory but how expression of Bdnf in the brain is influenced by truly acute bouts of exercise is currently unknown. Understanding the temporal dynamics of Bdnf expression in response to acute exercise offers the opportunity to optimize acute exercise prescription to enhance brain plasticity. In addition, *in vitro* and *ex vivo* exposure of hippocampal neurons to hormones and neurotrophins known to be upregulated during acute exercise regulates phosphorylation and trafficking of the GluR1 subunit of the AMPA-type glutamate receptor (AMPAR), but the ability for exercise itself to initiate these pathways in the hippocampus has not been studied. Trafficking of the AMPA-type ionotropic glutamate receptor (AMPAR) to and from the synapse is crucial for synaptic plasticity at excitatory synapses. Activity-dependent receptor trafficking is mediated by phosphorylation of the GluR1 subunit of the AMPAR and can be engaged by physiologically arousing stimuli (e.g. emotional stimuli, catecholamines), which in turn reduces the threshold for long-term potentiation (LTP) and enhances learning and memory. Exercise is a non-invasive, practical stimulus with the potential to engage this critical molecular mechanism to enhance synaptic plasticity and learning. Short-term exercise training also reduces the threshold for LTP, a mechanism of activity-dependent synaptic plasticity known to contribute to many forms of memory formation, but the pathway that links exercise to enhanced synaptic plasticity is not understood.

Exercise activates the “fight or flight” response, which is characterized by an elevation in peripheral and central catecholamines. Peripheral catecholamines (epinephrine and norepinephrine) activate the central noradrenergic system causing the release of norepinephrine in the brain. Norepinephrine signaling is a potent memory enhancer and strongly influences plasticity in multiple brain regions, including the hippocampus. The influence of exercise on peripheral epinephrine and central norepinephrine might explain how exercise, an activity that primarily influences peripheral tissues, has such a strong effect on hippocampal plasticity. However, catecholamine signaling and central noradrenergic activation are also associated with anxiety and anxiety-like behavior. If acute exercise is to be used as a non-pharmacological approach to enhance memory and mental health, understanding the behavioral response is essential, especially in rodents, which are commonly used to investigate how exercise influences brain plasticity and behavior. Chapter Four contains a manuscript that reports the experiments undertaken to test the hypotheses of Specific Aims 2 and 3. I utilized a truly acute bout of treadmill exercise by familiarizing mice to the treadmill without any running activity. Mice were exposed to behavioral testing or sacrificed immediately after the acute bout of treadmill exercise.

Aim #2: Determine the effect of acute exercise and exercise intensity on GluR1 phosphorylation, the expression of specific plasticity-associated genes, and novel object location memory in three-month old C57BL/6J mice.

Hypothesis #2: Acute treadmill exercise will increase GluR1 phosphorylation at sites critical for GluR1 membrane trafficking in an intensity-dependent manner, with greater phosphorylation in response to high- compared to moderate-intensity running. Acute exercise will also increase plasticity-associated gene expression and novel object location memory performance in an intensity-dependent manner.

Aim #3: Determine if acute high-intensity exercise increases anxiety-like behavior in the open field task and if this behavioral phenotype is attenuated with pre-treatment with the selective noradrenergic neurotoxin DSP-4.

Hypothesis #3: An acute bout of high-intensity exercise will increase anxiety-like behavior in the open field task.

Exploratory Hypothesis #3. The exercise-induced anxiety-like behavior will be attenuated in mice treated with DSP-4.

We observed that acute forced treadmill exercise does not influence GluR1 phosphorylation of the AMPAR but does increase expression of the rapidly transcribed *Bdnf* transcript IV. *Bdnf* is a complex gene producing up to 22 possible transcripts. These and previously reported data support the conclusion that *Bdnf IV* is sensitive to numerous stimuli, including exercise, and is rapidly transcribed. I also observed that acute exercise did not improve memory in a one-trial spatial memory task. The lack of improvement was likely due to anxiety-like behavior during the task, as I observed anxiety-like behavior following exercise in a locomotor-dependent anxiety task following exercise that was not rescued by lesioning central noradrenergic signaling. This behavior might have

been due to other signaling factors (e.g. dopaminergic, glucocorticoid, amygdalar activity, etc.) or fatigue following exercise.

Though this dissertation sought to address how a truly acute bout of exercise influences markers of hippocampal plasticity, it is important to understand acute exercise in the context of regular physical activity. Physical activity is important for physical and mental health, and it is therefore encouraged that individuals perform regular physical activity or purposeful exercise. So, if acute exercise is to be effectively utilized to enhance brain health and memory capability, it must be understood how acute and chronic exercise interact and the mediating mechanisms of a potential interaction. Chapter Five contains a manuscript describing the approach, results, and interpretation of a series of experiments designed to test the hypotheses for Specific Aims 4 and 5.

Aim #4: Determine if chronic exercise influences the effect of acute exercise on GluR1 protein phosphorylation and mRNA expression of plasticity-associated genes.

Hypothesis #4: Because chronic exercise will increase baseline levels of GluR1 phosphorylation and plasticity-associated gene expression, chronic voluntary wheel running will minimize *the response* to acute exercise. This will minimize the absolute difference in the level of GluR1 phosphorylation and plasticity-associated gene expression in mice that received chronic exercise + acute exercise versus acute exercise alone. However, the relative response to acute exercise will be lower in chronically exercise-trained mice.

Aim #5: Determine the influence of acute exercise and locus coeruleus noradrenergic signaling on specific *Bdnf* transcript expression.

Hypothesis #5: Acute exercise-induced expression of *Bdnf IV* will be dependent on noradrenergic signaling and will be attenuated by DSP-4. Acute exercise-induced expression of total *Bdnf* mRNA will persist following DSP-4 treatment due to increased expression of other *Bdnf* transcripts.

Mice were housed for one month with either freely rotating or locked running wheels before exposure to an acute bout of forced treadmill exercise. Though previous research suggests enhanced capacity to secrete catecholamines following exercise training, animals and humans show a reduced stress response to psychological and physical stress following exercise training. We observed that both acute and chronic exercise increased *Bdnf* mRNA expression but chronic exercise blunted the influence of acute exercise on *Bdnf IV* mRNA expression. We then investigated if noradrenergic signaling influences transcription of multiple *Bdnf* transcripts and observed that compromising noradrenergic signaling reduced *Bdnf IV* mRNA expression but this was rescued with acute exercise, contrary to our original hypothesis that compromising noradrenergic signaling would attenuate the acute exercise-induced increase in this transcript. Though compromising noradrenergic signaling reduced *Bdnf IV* expression, it increased *Bdnf VI* expression, demonstrating the remarkable complexity of *Bdnf* gene transcription and the influence of noradrenergic signaling.

This dissertation begins with a review of literature (Chapter Two) examining the influence of acute and chronic exercise on mental health and the structural and functional brain plasticity observed in humans and rodents. The review of literature is followed by three chapters in manuscript form (Chapters Three, Four, and Five) that describe the methodological approaches, results, and interpretations of the dissertation experiments. Finally, Chapter Six summarizes the findings of all three studies and discusses implications and future directions for this area of research.

Chapter 2. *Review of Literature*

Introduction

Mental health disorders are an increasing health burden in the United States and around the world. Disorders such as anxiety, depression, post-traumatic stress disorder (PTSD), and age-related dementias (e.g. Alzheimer's disease) are debilitating conditions that not only impact the suffering individual, but also family, friends, and the economy. The National Institute of Mental Health claims that 6.7% of U.S. adults experienced an episode of major depression in 2014 (Center for Behavioral Health Statistics Quality, 2015) and others have reported lifetime prevalence of 28.8% and 6.8% for anxiety disorders and PTSD, respectively (Kessler *et al.*, 2005). Moreover, in the 2014-2015 Alzheimer's Progress Report, the National Institute of Aging stated that roughly five million Americans are currently living with Alzheimer's disease, and with the growing aging population and lack of current therapies, this number is expected to increase (National Institute on Aging, 2015). These mental health disorders span the age spectrum and negatively impact the lives of millions of young and aged individuals. An abundance of research suggests that exercise is a potential non-invasive therapeutic technique that could be used to treat and defend against anxiety, depression, PTSD, and age-related dementias. Research is still needed to understand the most effective exercise regimens, the mechanisms of action, and sensitive brain regions mediating the beneficial adaptations to best treat and/or prevent these debilitating conditions. Exercise may serve as a stand-

alone treatment or be used in combination with medications and cognitive therapies. Moreover, in addition to improving the mental health of patient populations, exercise has the potential to improve cognitive function in healthy individuals. Effective exercise strategies may be used to accelerate and strengthen learning in students of all ages and improve training practices in corporate, military, and government agencies.

Though multiple brain regions influence and/or are affected by mental health disorders, the hippocampal formation is linked to the development and/or progression of many disorders affecting Americans today such as anxiety, depression, PTSD, schizophrenia, and age-related dementias (Pajonk *et al.*, 2010; Small *et al.*, 2011). Both structural and functional changes coincide with disease progression, but remarkably, this structure is also highly sensitive to physical activity and exercise training (Voss *et al.*, 2013), highlighting the therapeutic potential of exercise to treat these mental health disorders. The hippocampus is a brain structure important for the formation of episodic and spatial memories and plays an important role in emotional regulation, containing a high content of stress hormone receptors (Osborne *et al.*, 2015). It mediates these cognitive functions via a unique gradient along the longitudinal axis of the structure. Using lesioning techniques in rodents and fMRI in humans, researchers have identified that the dorsal (posterior in humans) region is particularly involved in spatial navigation while the ventral (anterior in humans) region is principally involved in emotional responses. This functional delineation along the longitudinal axis is consistent with the cortical and subcortical

connections found along the longitudinal axis (Strange *et al.*, 2014). For example, the fear and emotional center of the brain, the amygdala, and the infralimbic and prelimbic cortices (cingulate areas involved in emotional regulation) are more connected with the ventral hippocampus while information from the visual cortex projects primarily to the dorsal/posterior hippocampus (Amaral & Witter, 1989). Further support for a longitudinal gradient of function comes from identification of unique transcriptional profiles along the longitudinal axis (Thompson *et al.*, 2008; Dong *et al.*, 2009). Importantly, exercise influences both the dorsal and ventral hippocampus (Schoenfeld *et al.*, 2013). In addition to the longitudinal axis, the hippocampus contains unique subfields (dentate gyrus → CA3 → CA1) that are uniquely sensitive to disease and environmental stimuli (McGaugh & Roozendaal, 2002; Small *et al.*, 2011). Exercise training leads to structural adaptations throughout the hippocampal subfields (Eadie *et al.*, 2005; Stranahan *et al.*, 2007; Lin *et al.*, 2012) and can serve as an intervention to improve the functional and structural integrity of this brain region that is so critical for cognitive and emotional health.

This review of literature will provide a detailed analysis of the research in the field of physical activity/exercise training on brain health and function. I will focus on how exercise influences the human and rodent hippocampus, highlighting structural and functional adaptions and the mechanisms mediating these effects. Consistent with the order of experiments in the dissertation, this literature review will begin with a review of studies examining the use of chronic exercise to influence brain health, then will transition to studies of acute exercise

(or acute psychological stress as a surrogate for acute exercise), and will finish with a review of literature reporting potential interactions between acute and chronic exercise.

Physical Activity is Beneficial for Healthy Cognitive Aging

Exercise is an excellent environmental stimulus for maintaining brain health into old age. Large-scale epidemiological studies have shown that higher levels of chronic physical activity are associated with reduced risk of cognitive decline and age-related dementias such as Alzheimer's disease (Laurin *et al.*, 2001; Yaffe *et al.*, 2001; Weuve *et al.*, 2004; Rovio *et al.*, 2005; Larson, 2006; Angevaren *et al.*, 2008; Middleton, 2011; Buchman *et al.*, 2012). Though many of the epidemiological studies used very general methods of physical activity (e.g. self-reported physical activity) and/or cognitive functioning assessment (e.g. Mini-Mental State Exam), they offered powerful evidence for the benefit of exercise on brain health and provided the stimulus for more systematic investigations of exercise and brain health/plasticity that have followed. Larson et al. (2006) investigated the relationship between self-reported physical activity and rates of dementia over an average of 6.2 years in 2,581 cognitively intact men and women aged 65 years and older. They found that individuals who exercised three or more times per week had 32% reduced odds of dementia. Similarly, in a group of 16,466 women aged 70 years and older, researchers found that women who participated in the greatest amount of leisure time physical activity (assessed via biennial questionnaires) were 20% less likely to be cognitively

impaired when compared to women who participated in the least physical activity (Weuve *et al.*, 2004). Consistent with these relatively short follow-up periods, another investigation reported that individuals who participated in physical activity two or more times per week had 52% lower odds of dementia 21 years later (Rovio *et al.*, 2005). These earlier studies that used self-reported physical activity have been supported by more advanced measures of energy expenditure and activity, such as doubly labeled water, indirect calorimetry (Middleton, 2011), and actigraphy (Buchman *et al.*, 2012). Though not all investigations have reported beneficial effects of self-reported physical activity on the risk of developing dementia (Rovio *et al.*, 2007), the literature provides exceedingly strong evidence to support that chronic physical activity is an effective way to reduce cognitive decline and age-related cognitive impairments. It is important to note that these longitudinal studies have demonstrated that physical activity performed in early or mid-life predicts cognitive functioning later in life (Sofi *et al.*, 2010). Remarkably, an investigation by Middleton et al. (2010) showed that self-reported physical activity during the teenage years was most predictive of cognitive aging in elderly women. Women who were physically inactive during their teenage years could reduce their risk of cognitive impairment by becoming active during their 30s and 50s but there was no additional benefit of becoming active in their 30s and 50s if the subjects were already active during the teenage years (Middleton *et al.*, 2010). Taken together, the literature on cognitive aging informs the importance of understanding the most effective exercise strategies that can be performed throughout the lifespan to maintain brain health into old

age. The structural adaptations occurring in the human and rodent brain with exercise training and the mechanisms responsible have received considerable attention from researchers, as these adaptations likely mediate the cognitive benefit of regular physical activity and risk of age-related cognitive impairment and dementias.

Physical Activity/Exercise Training Increases Brain Volume

The maintenance of cognitive function with physical activity is potentially due to the remarkable preservation of brain tissue volume observed in physically active and/or highly fit adults compared to sedentary adults (Colcombe *et al.*, 2003; Erickson *et al.*, 2009; 2010). This is important as the aging process is associated with a loss of gray and white matter tissue volume (Resnick *et al.*, 2003) and a decrease in hippocampal volume (Ylikoski *et al.*, 2000), which has been reported to occur more rapidly than in other cortical areas (Jernigan *et al.*, 2001). Intervention studies have provided strong support for the association of physical activity and brain tissue volume by demonstrating that tissue volume actually increases with physical activity. Colcombe et al. (2006) reported that six months of aerobic exercise training increased gray and white matter volume in individuals aged 60-79 years. These researchers did not investigate the association between increased tissue volume and cognitive function. Erickson et al. (2011) showed that one year of structured aerobic exercise training in older adults increased the size of the human anterior hippocampus by approximately 2%. Importantly, control subjects in this study showed a reduction in

hippocampal volume of approximately 1.4% over the same 12-month intervention period. This shows remarkable structural plasticity in the adult brain as exercise not only prevented age-related tissue loss but also increased tissue volume. While the aerobic exercise group in this study did not perform better on a spatial memory task compared to the stretching control group, cardiorespiratory fitness and changes in hippocampal volume were associated with spatial memory performance before and after the intervention (Erickson *et al.*, 2011). Increased hippocampal volume has also been reported with six months of aerobic exercise training in women aged 70-80 years with suspected mild cognitive impairment (Brinke *et al.*, 2015).

The aged population has received the most attention in the field of physical activity and brain health since there is generally greater variability in cognitive function in this group compared to young adults who have higher and more stable cognitive function (Voss *et al.*, 2011). The limited research in young and middle-aged adults has provided conflicting evidence for the structural and functional benefits of regular physical activity or exercise training in these age groups (Prakash *et al.*, 2015); however, Pereira et al. (2007) showed that three months of aerobic exercise training increased cerebral blood volume (which the authors suggest is an imaging “correlate” of neurogenesis) in the hippocampi of adults aged 21-45 years (mean age 33 yrs). The authors also reported an association between cardiorespiratory fitness and learning on a verbal memory task and a trend for improved delayed recall after exercise training. This study shows that structural and functional adaptations to exercise training are not

limited to the old. Overall, the literature reveals that the human hippocampus displays remarkable structural plasticity in response to regular physical activity in both young and old adults. Research in rodents has furthered our understanding of these structural changes and the critical mechanisms of structural plasticity.

Physical Activity and Structural Adaptations in Rodents

Exercise induces structural adaptations in the rodent hippocampus. Rodent research has definitively demonstrated that physical activity is effective in stimulating adult neurogenesis in the dentate gyrus of the hippocampus (Voss *et al.*, 2013). In addition to increasing the number of neurons in the dentate gyrus, physical activity increases dendritic branching and synaptogenesis throughout the hippocampus (Eadie *et al.*, 2005; Redila & Christie, 2006; Stranahan *et al.*, 2007; Lin *et al.*, 2012). Lin et al. (2012) showed that four weeks (5 d/wk) of forced treadmill running or voluntary wheel running increased the dendritic field and spine density in the CA3 region of the rat hippocampus. Another study showed that two months of voluntary wheel running increased dendritic spine density in granule cells of the dentate gyrus and pyramidal cells in CA1 and layer III of the entorhinal cortex (Stranahan *et al.*, 2007). These structural adaptations are most likely responsible for the observed increase in hippocampal size following an aerobic exercise intervention (Pereira *et al.*, 2007) and are consistent with the numerous functional and behavioral adaptions observed following exercise training and physical activity such as enhanced hippocampal-dependent learning and memory (Gomez-Pinilla & Hillman, 2013; Voss *et al.*,

2013), hypothalamus-pituitary-adrenal (HPA) axis regulation (Stranahan *et al.*, 2008), and reduced anxiety- and depression-like behavior (Sciolino & Holmes, 2012; Holmes, 2014). These hippocampal adaptations demonstrate the sensitivity of this brain region to exercise training and physical activity, consistent with adaptations observed in human studies. Moreover, these structural adaptations are due to numerous signaling mechanisms that occur with physical activity. Thoroughly understanding these signaling mechanisms will greatly enhance the ability to maintain and improve brain health with exercise and other "exercise-like" interventions. Brain derived neurotrophic factor (BDNF) has been identified as a critical neurochemical for normal brain health, structural plasticity, behavioral adaptations, and exercise induced-enhancement of brain health. The importance of BDNF in normal brain health and exercise induced hippocampal plasticity will be discussed in detail over the next several sections of this review.

Brain-Derived Neurotrophic Factor Is Essential for Structural and Functional Hippocampal Plasticity

Research has identified a number of potential mechanisms that might mediate the effect of chronic exercise on hippocampal structure and function. Elevated expression (mRNA and/or protein) and downstream signaling of BDNF has been identified as a primary exercise-induced regulator of functional and structural plasticity. BDNF is a critical factor in the maintenance of optimal brain health and plays an integral role in functional and structural plasticity in the hippocampus throughout the lifespan. It is a remarkable signaling protein of the

neurotrophin family, a group of structurally related proteins that are important for neural growth and development (Poo, 2001). BDNF is important for structural adaptations such as hippocampal neurogenesis (Lee *et al.*, 2002; Rossi *et al.*, 2006; Sairanen, 2005; Scharfman *et al.*, 2005; Taliaz *et al.*, 2009) and dendritic/synaptic development (Alonso *et al.*, 2004; Bergami *et al.*, 2008; Bohlen und Halbach *et al.*, 2008), as well as functional adaptations at the synaptic (Korte *et al.*, 1995; Figurov *et al.*, 1996; Korte *et al.*, 1996; Patterson *et al.*, 1996; Kang *et al.*, 1997; Ma *et al.*, 1998; Chen *et al.*, 1999; Zakharenko *et al.*, 2003) and behavioral levels (Linnarsson *et al.*, 1997; Ma *et al.*, 1998; Mu *et al.*, 1999; Mizuno *et al.*, 2000; Alonso *et al.*, 2002; Heldt *et al.*, 2007; Bekinschtein *et al.*, 2008). Using RNA interference (Taliaz *et al.*, 2009) and genetic manipulations to knockout (KO) or knockdown *Bdnf* (Rossi *et al.*, 2006) or its receptor (*TrkB*) (Bergami *et al.*, 2008), researchers have demonstrated that Bdnf signaling is critical for adult neurogenesis to occur under normal conditions (Taliaz *et al.*, 2009) or following enriched housing conditions (Rossi *et al.*, 2006). Importantly, though TrkB also binds neurotrophin-4 (NT4), another member of the neurotrophin family, Rossi et al. (2006) demonstrated that *Nt4* KO mice showed normal neurogenesis following environmental enrichment, whereas *Bdnf* heterozygous knockdown mice had significantly reduced hippocampal neurogenesis.

Remarkably, neurogenesis can be induced by exogenous delivery of Bdnf (Scharfman *et al.*, 2005). Bergami et al. (2008) used a *TrkB* inducible KO mouse model to demonstrate that TrkB signaling (and presumably Bdnf signaling) is

necessary for development of newborn neurons in adult mice. *TrkB* KO mice also showed reduced dendritic and spine growth, which is consistent with other studies that have reported that Bdnf-TrkB signaling is necessary for dendritic spine density in the hippocampus (Alonso *et al.*, 2004; Bohlen und Halbach *et al.*, 2008). In fact, exogenous Bdnf delivery increases dendritic spine density in mature hippocampal neurons in culture (Tyler & Pozzo-Miller, 2001; Ji *et al.*, 2010). The newborn neurons from TrkB KO mice in the Bergami et al. (2008) investigation also displayed reduced capacity for long-term potentiation (LTP), a cellular model for memory. This is consistent with other studies that have demonstrated that Bdnf-TrkB signaling is critical for inducing synaptic plasticity. Korte et al. (1995) deleted the *Bdnf* coding sequence in mice to create heterozygous knockdown and homozygous KO mice and observed impaired long-term potentiation (LTP) in CA3-CA1 synapses. Only approximately 30% of slices from knockdown and KO mice displayed LTP following tetanus stimulation compared to approximately 87% from wild type mice showing successful LTP. Of note, when LTP did occur in the mutant mice, it displayed lower amplitude and a rapid decline in amplitude, suggesting that maintenance of LTP was also significantly impaired in these mice. Other investigations have shown that Bdnf is important for both the early and late phases of LTP (Figurov *et al.*, 1996; Patterson *et al.*, 1996; Kang *et al.*, 1997). Korte et al. (1996) later demonstrated that re-expressing Bdnf in CA1 neurons (*ex vivo*) with an adenovirus rescued the impaired LTP observed in the Bdnf-mutant mice. Other studies have used various approaches to block Bdnf availability (Ma *et al.*, 1998; Chen *et al.*, 1999)

or signaling (Figurov *et al.*, 1996; Minichiello *et al.*, 2002) to demonstrate its importance in LTP. Notably, the impaired LTP observed in slices from *Bdnf* KO animals can be rescued with recombinant Bdnf (Patterson *et al.*, 1996).

In light of the evidence of impaired neurogenesis, compromised neuronal structure, and reduced plasticity following treatments that reduce Bdnf availability or signaling, it is no surprise that these treatments also have deleterious effects on memory (Linnarsson *et al.*, 1997; Ma *et al.*, 1998; Mu *et al.*, 1999; Mizuno *et al.*, 2000; Alonso *et al.*, 2002; Heldt *et al.*, 2007; Bekinschtein *et al.*, 2008). Reducing Bdnf availability impairs learning and/or memory in the novel object recognition task (Heldt *et al.*, 2007), Morris water maze (Linnarsson *et al.*, 1997; Mu *et al.*, 1999), non-swimming maze tasks (Mizuno *et al.*, 2000), and one-trial avoidance tasks (Ma *et al.*, 1998; Alonso *et al.*, 2002). It is important to recognize that learning itself is associated with increased expression of Bdnf (Ma *et al.*, 1998; Mizuno *et al.*, 2000; Alonso *et al.*, 2002; Bekinschtein *et al.*, 2008; Lubin *et al.*, 2008), and remarkably, Bdnf availability is important for both short- and long-term memory formation and persistence. Bekinschtein et al. (2008) investigated the role of Bdnf in long-term memory formation/persistence and observed that Bdnf was necessary and sufficient for protein synthesis-dependent long-term memory persistence. Infusion of anisomycin, a protein synthesis inhibitor, into the hippocampus 12 hours after a one-trial inhibitory avoidance paradigm reduced memory performance seven days but not two days after training, suggesting an impairment of long-term memory retention but not formation. Infusion of a recombinant Bdnf protein 15 minutes after the

anisomycin infusion rescued memory persistence. Further, a weak inhibitory avoidance training paradigm that was unable to induce Bdnf expression and long-term memory persistence resulted in long-term memory persistence when coupled with infusion of a recombinant Bdnf. Alonso et al. (2002) used an anti-Bdnf antibody infused into CA1 of the dorsal hippocampus to demonstrate that Bdnf is essential for short-term and long-term memory performance in an inhibitory avoidance task. When the anti-Bdnf antibody was infused 15 minutes prior to the training trial, performance was impaired 1.5 and 24 hours later. Interestingly, when the antibody was delivered one or four hours after training, performance 24 hours later was impaired but not when it was delivered at 0 or 6 hours post-training. Infusion of recombinant Bdnf 15 minutes before or immediately after training improved short-term memory (1.5 hrs), while infusion at 1 or 4 hours post training improved long-term memory (24 hrs). These data suggest that there are critical windows in time in which Bdnf expression is crucial for the formation of short- and long-term memory.

Research on BDNF has provided profound evidence of the significance of this neurotrophin in the maintenance and/or enhancement of brain health. Aging, obesity, diabetes, and numerous mental health disorders such as depression, anxiety, and Alzheimer's disease are associated with reduced BDNF levels, which may contribute to memory impairments (Marosi & Mattson, 2014). Exercise training is an effective way of increasing BDNF expression in healthy and pathological populations (Cotman & Berchtold, 2002; Cotman *et al.*, 2007). Moreover, BDNF is essential for many of the beneficial adaptations observed

following exercise training; however, many questions about exercise and Bdnf expression still remain. For example, what are the most effective exercise strategies to increase BDNF expression and what signaling mechanisms induced by exercise stimulate BDNF expression? Though research has established that maintaining a certain level of BDNF expression is important for brain health, the studies by Bekinschtein et al. (2008) and Rossi et al. (2006) reveal that certain "windows of opportunity" exist for BDNF expression to influence memory. Once questions such as these are adequately addressed, exercise training can be tailored to most effectively increase BDNF expression to enhance brain health in healthy and pathological populations.

Exercise Training/Fitness and Peripheral BDNF Levels

Generally, it is believed that exercise training is associated with increased circulating BDNF, though research in healthy adults has provided inconclusive evidence with some studies showing higher basal BDNF concentrations following training or in highly fit individuals (Zoladz *et al.*, 2008; Erickson *et al.*, 2011), no difference (Castellano & White, 2008; Schiffer *et al.*, 2009; Flöel *et al.*, 2010), or even lower BDNF in trained or highly fit individuals (Chan *et al.*, 2008; Nofuji *et al.*, 2008; Currie *et al.*, 2009; Babaei *et al.*, 2014). These discordant findings are potentially due to differences in sample sizes, training status of the participants, types of exercise training protocols, or whether BDNF was measured in serum or plasma (Knaepen *et al.*, 2010). Moreover, the biological importance of circulating BDNF levels for hippocampal health is not understood. Erickson et al. (2011)

demonstrated that the increase in hippocampal volume following six months of aerobic exercise training was associated with increased serum BDNF. An association between peripheral BDNF levels and anterior hippocampal volume was also observed by Wagner et al. (2015); however, they actually observed a reduction in hippocampal volume following a six-week exercise training intervention that improved fitness. Higher levels of BDNF were still associated with greater hippocampal volume even though training was associated with reduced hippocampal volume. It is curious that both fitness and BDNF are associated with hippocampal volumes, yet numerous studies indicate that higher fit individuals have lower peripheral BDNF levels. This questions the usefulness of using peripheral BDNF levels to infer adaptations occurring in the hippocampus. Currently, it is necessary to use animals to characterize the influence of exercise on hippocampal Bdnf and the mediating mechanisms and associated adaptations.

Physical Activity Increases Hippocampal Bdnf in Rodents

In the rodent hippocampus, Bdnf protein and mRNA are elevated following both short-term (≤ seven days; Neeper *et al.*, 1995; 1996; Molteni *et al.*, 2002; Vaynman *et al.*, 2003; Berchtold *et al.*, 2005; Huang *et al.*, 2005; Ding *et al.*, 2011; Sartori *et al.*, 2011) and longer exercise exposures (> seven days; Molteni *et al.*, 2002; Farmer *et al.*, 2004; Berchtold *et al.*, 2005; Liu *et al.*, 2009; Berchtold *et al.*, 2010; Ding *et al.*, 2011; Kobilo *et al.*, 2011; Sartori *et al.*, 2011; Marlatt *et al.*, 2012; Wrann *et al.*, 2013). Importantly, studies show that both forced

treadmill exercise and voluntary wheel running increase hippocampal expression of Bdnf (Liu *et al.*, 2009; Alomari *et al.*, 2013). It is well established that exposure to physical activity in rodents improves spatial (Fordyce & Farrar, 1991; Vaynman *et al.*, 2004; Gomez-Pinilla *et al.*, 2008; Liu *et al.*, 2009; Creer *et al.*, 2010; Intlekofer *et al.*, 2013) and non-spatial (O'Callaghan *et al.*, 2007; Liu *et al.*, 2008; 2009) memory; however, blocking Bdnf-TrkB signaling (Vaynman *et al.*, 2004; Gomez-Pinilla *et al.*, 2008; Intlekofer *et al.*, 2013) prevents such beneficial adaptations to exercise training, including improved spatial memory and elevated expression of plasticity associated markers. Using a recombinant human TrkB-IgG chimera to block Bdnf action, Vaynman et al. (2004) demonstrated that Bdnf signaling was necessary for the improvement in Morris water maze performance afforded by one week of voluntary wheel running. Importantly, the chimera alone did not impair task performance; it merely attenuated the beneficial effect of voluntary exercise. The chimera also blocked the exercise-induced increase in CREB phosphorylation and mRNA expression of *Bdnf*, *TrkB*, *Synapsin 1*, and *Creb*. This group later replicated the finding that one week of voluntary exercise improved performance on the Morris water maze and that this was blocked using the TrkB-IgG chimera. They further extended the findings of their previous report to show that blocking Bdnf signaling attenuated the metabolic transcriptional profile induced by one week of voluntary running (Gomez-Pinilla *et al.*, 2008). Lin et al. (2012) reported that injecting a TrkB inhibitor, K252A, into the dorsal hippocampus prevented the exercise facilitation of contextual fear learning, a hippocampal-dependent fear learning task. Unfortunately, K252A might block

activity of other important kinases, such as CAMKII, and therefore, it is difficult to attribute this finding solely to Bdnf-TrkB signaling. Other researchers have attempted to mimic the effects of exercise using intracerebral injections of a human recombinant Bdnf and observed a similar improvement in spatial learning between mice exposed to seven days of forced treadmill running and the mice injected with the recombinant Bdnf (Griffin *et al.*, 2009; Bechara *et al.*, 2014). These mimicking studies only demonstrate that the effects of exercise and infusions of recombinant Bdnf have similar effects on spatial learning but do not provide evidence that Bdnf is mediating the effect of exercise. The mimicking investigations would be stronger if the recombinant Bdnf infusion rescued compromised exercise-induced facilitation of learning by a manipulation that reduces endogenous Bdnf availability. Other mRNA and protein targets such as tissue-type plasminogen activator (tPa), insulin like growth factor (Igf-1), and peroxisome proliferator-activated receptor γ coactivator 1 alpha (Pgc-1a) have all been shown to be critical for exercise-induced adaptations in the rodent hippocampus and remarkably, each of these proteins is linked to Bdnf signaling or expression (Vaynman *et al.*, 2004; Ding *et al.*, 2006; 2011; Voss *et al.*, 2013; Wrann *et al.*, 2013).

An interesting caveat to the research examining exercise training and Bdnf expression in rodents is that the majority of research has focused on exercise exposures from seven to 28 days. In fact, “chronic” or “long-term” exercise has not been adequately defined in the literature. Importantly, wheel running volume declines over time (Richter *et al.*, 2014; Venezia *et al.*, 2015) and it is possible

that the high levels of running and novelty of the new environment upon introduction of a running wheel stimulate Bdnf expression to a level that is not observed following prolonged exposure to a wheel. However, there is some evidence of elevated Bdnf expression with longer exercise exposures. Ninety days of wheel running was effective at increasing hippocampal Bdnf protein expression in rats (Berchtold *et al.*, 2005). Eight months of wheel (Marlatt *et al.*, 2012) and treadmill (O'Callaghan *et al.*, 2007) running have resulted in greater Bdnf expression in older animals; however, aging is associated with a decrease in Bdnf expression (O'Callaghan *et al.*, 2009) and the benefits of long-term exercise training on Bdnf expression in these investigations may be a rescue of compromised Bdnf expression due to aging in lifelong sedentary conditions. Whether similar benefits would be observed in young adult rodents is not known.

Sex Differences in Exercise Induced BDNF Expression

Research in both humans and animals suggests a complex relationship between sex hormones and Bdnf expression (Carbone & Handa, 2013; Pluchino *et al.*, 2013). The menstrual cycle influences peripheral levels of BDNF and women with normal ovulatory cycles have higher levels of BDNF compared to amenorrhoeic or postmenopausal women (Begliuomini *et al.*, 2007). Hormone therapy used for male-to-female transsexuals is associated with reduced peripheral BDNF (Fuss *et al.*, 2015). Even diurnal fluctuations in peripheral BDNF differ between sexes, with men experiencing greater variation throughout the day (Piccinni *et al.*, 2008; Choi *et al.*, 2011). Concerning exercise and BDNF

in humans, a recent meta-analysis reported that effect sizes were generally smaller in studies that included females (Szuhany *et al.*, 2015) and in a systematic review, Knaepen et al. (2010) suggest that sex might be a contributing factor to differences observed between studies reporting the influence of exercise on peripheral BDNF.

A limited number of studies have utilized both male and female animals in investigations on the influence of exercise on hippocampal Bdnf expression. Berchtold et al. (2001) reported that five days of wheel exposure increased hippocampal Bdnf mRNA and protein expression in female rats; however, in rats that were ovariectomized seven weeks before exercise exposure, exercise was unable to increase hippocampal Bdnf mRNA or protein. Ovariectomy itself reduced hippocampal Bdnf mRNA and protein. Gallego et al. (2015) reported an increase in hippocampal Bdnf mRNA and protein expression following 21 days of voluntary wheel exposure in adolescent male and female mice while Titterness et al. (2011) reported no differences in Bdnf protein in male or female adolescent rats. However, Titterness et al. (2011) also reported that exercise facilitated synaptic plasticity (i.e. LTP) only in adolescent males, which is surprising in the absence of increased Bdnf expression. Understanding sex differences in response to environmental factors and therapeutic treatments is very important for optimizing brain health (Cahill, 2006) and much remains unknown about how sex and exercise interact to enhance critical signaling factors such as BDNF. For example, as males generally display diurnal variation in BDNF levels, potentially there is an optimal time to participate in exercise activities to effectively increase

BDNF levels. Moreover, in females, exercise participation could be tailored to the menstrual cycle or hormone replacement therapies.

Transcription of the BDNF Gene

Age, sex, and exercise type, duration, and intensity all influence BDNF availability through intricately controlled transcription of the highly complex *BDNF* gene. Total *Bdnf* mRNA expression is the result of the combined expression of multiple *Bdnf* transcripts. The *Bdnf* gene was originally characterized as having four non-coding exons (I, II, III, IV) that alternatively splice to one 3' protein coding exon (V). However, in 2007, the gene was re-characterized and found to have eight non-coding exons [I, II, III (new), IV (previously III), V (new), VI (previously IV), VII (new), VIII (new)] with individual promoters that all splice to one 3' protein coding exon (exon IX; previously V) (Aid *et al.*, 2007). In addition, the gene has two 3' polyadenylation sites (Timmusk *et al.*, 1993; Aid *et al.*, 2007). This complexity results in as many as 22 possible transcripts (Zheng *et al.*, 2012). Interestingly, all *Bdnf* transcripts are translated into the same protein (proBDNF), which is then cleaved to produce the mature plasticity-associated protein. Though it is not fully understood why so many transcripts are needed to produce the same protein, the transcripts have different subcellular localization and transport capabilities (Baj *et al.*, 2011; 2013) and the large number of transcripts offers tight temporal and regional control of transcription, mRNA survival, and distribution. For example, Baj et al. (2013) reported a unique spatial code of *Bdnf* transcripts throughout the hippocampal subfields under rest, with *Bdnf VI*

being the primary transcript found in the dendrites of all hippocampal subfields; however, after pilocarpine-induced neuronal activity, *Bdnf* transcripts IV and VI were highly present in hippocampal dendrites. *Bdnf IV* was originally believed to be restricted to the soma even with neuronal activity (Chiaruttini *et al.*, 2008) but the more recent study by Baj et al. (2013) demonstrated that this transcript is transported to the dendrites following high neuronal activity. *Bdnf transcript IV* (driven by promoter IV) has been the target of much of the research on *Bdnf* transcription. Though *Bdnf* is considered to be an activity-regulated gene, promoter IV-driven *Bdnf* transcription is especially sensitive to neuronal activity (Tao *et al.*, 1998; 2002; Martinowich *et al.*, 2003). Intense investigation of transcriptional regulation of exon IV has revealed this transcript to be calcium sensitive, containing at least three calcium-responsive elements around the transcriptional initiation site of exon IV (Zheng *et al.*, 2011; 2012). While *in vitro* work demonstrates a clear association between neuronal activity and *Bdnf IV* transcription (Tao *et al.*, 1998; Martinowich *et al.*, 2003), environmental stimuli *in vivo* result in additional *Bdnf* transcript-specific transcription. Using a contextual fear conditioning paradigm in rats, Lubin et al. (2008) demonstrated that hippocampal total *Bdnf* mRNA (exon IX) was elevated after both context exposure alone (no shock) and associative fear conditioning, though the elevated expression of total *Bdnf* was mediated by different exon-containing transcripts. Following context exposure alone, the elevated total *Bdnf* mRNA was due to elevated *Bdnf I* and *VI* mRNA, while after associative fear conditioning it was due to elevated *Bdnf IV* expression. Acute immobilization stress differentially

influences transcription of specific transcripts depending on the length of immobilization. Two hours of immobilization resulted in reduced total *Bdnf* (exon IX), *Bdnf I*, and *Bdnf IV* compared to non-stress controls (Fuchikami *et al.*, 2008). An earlier study by Marmigere et al. (2003) demonstrated that transcripts containing exons I, II, and IV (III in the paper) were elevated after short exposures to immobilization stress (15 – 60 minutes). *Bdnf IV* expression was elevated at 15 minutes, which suggests a very rapid stimulus-induced expression of this transcript, though it reached peak expression at 60 minutes. Transcripts I and II, were not yet elevated at 15 minutes but also reached peak expression at 60 minutes. Transcript VI expression was unchanged at 60 minutes and lower than controls at 180 minutes (Marmigère *et al.*, 2003). Concerning exercise training and transcript-specific *Bdnf* expression, three weeks of voluntary wheel running in C57BL/6J mice was sufficient to elevate mRNA for total *Bdnf* and exons I and IV in the hippocampus but did not increase exon VI expression (Intlekofer *et al.*, 2013). These changes in *Bdnf* transcription were associated with cognitive benefits in an object location task that were blocked by hippocampal infusion of a short-interfering RNA for *Bdnf*. A study by Zajac et al. (2009) reported that eight weeks of wheel running increased hippocampal expression of exons I, II, III, IV, and VI in females and exons I, II, and III in males. Baj et al. (2012) reported an increase in total *Bdnf* following 28 days of voluntary wheel running in the somata and apical dendrites of CA3. There was no effect of exercise on *Bdnf IV* expression but there was an increase in CA1 and CA3 *Bdnf VI* expression. In addition, *Bdnf* exon IV in the rodent hippocampus undergoes

epigenetic modifications with seven days of running wheel exposure, which suggested enhanced transcriptional capabilities (Gómez-Pinilla *et al.*, 2010).

These studies demonstrate that control of *BDNF* transcription is highly complex, with different environmental stimuli inducing unique transcriptional profiles in a region-, time-, and sex-dependent manner. Compromised *BDNF* expression influences a number of mental health disorders, so understanding the *BDNF* transcripts most influenced by these disorders and affected by common or novel treatment interventions will greatly enhance approaches to treating at-risk or suffering individuals. It is strongly supported that regular chronic physical activity is beneficial for overall health, though to truly capitalize on the benefits of exercise as a non-invasive therapeutic strategy for mental health disorders, it is critical to understand the adaptations occurring in response to acute bouts of exercise. A thorough understanding of the immediate and delayed adaptions to acute exercise can identify exercise strategies to be used most effectively to enhance mental health. For example, to improve cognitive function in cognitively impaired older adults or Alzheimer's disease patients, there may be an optimal approach of combining acute exercise bouts with memory training, or an optimal intensity of acute exercise combined with a pharmacological approach. Answering these types of questions will require thorough investigations of acute exercise and the adaptations and mediating mechanisms.

Acute Exercise and Memory

The impact of acute bouts of physical activity on the brain is less understood than chronic physical activity or exercise training. This may be due to the lack of acute exercise studies in rodents to identify mechanisms and structural adaptations. It is important to remember that the mechanisms mediating the effects of chronic physical activity or exercise training are likely the result of accumulated repeated bouts of acute exercise. Indeed, acute bouts of physical activity have been reported to improve cognitive performance in humans, though differences in exercise protocols, types of cognitive measures, and whether cognitive testing took place before, during, or after exercise make the data difficult to interpret (Brisswalter *et al.*, 2002; Tomporowski, 2003; Lambourne & Tomporowski, 2010; Chang *et al.*, 2012; Roig *et al.*, 2013). Much of the research to date has focused on acute exercise-induced arousal and cognitive processing *during* the acute bout of exercise, or focused on cognitive tasks designed to assess executive functioning. However, recent meta-analyses have provided support for an association between acute exercise and memory performance, especially when the cognitive task is performed after the exercise (Lambourne & Tomporowski, 2010; Chang *et al.*, 2012; Roig *et al.*, 2013). Using a meta-analytic approach, Lambourne and Tomporowski (2010) explored the influence of acute exercise timing relative to cognitive task performance. They determined that cognitive task performance was impaired when the task was performed during the first 20 minutes of acute exercise. However, task performance was facilitated when the task was performed after the first 20 minutes of exercise initiation. They also found an improvement in cognitive task

performance when the task was performed after the acute bout of exercise. Interestingly, they reported larger effects for memory performance compared to processing and/or reaction time following an acute bout of exercise. Concerning memory, Coles and Tomporowski (2008) reported that 40 minutes of exercise improved long-term memory on a delayed free recall test in a group of young adults. Individuals exercised for 40 minutes (30 minutes at 60% VO2max; 5 min-warm and 5 min cool-down) prior to the encoding phase of the memory task. Interestingly, the benefit of acute exercise was only observed for long-term memory, with no benefit observed for short-term memory. A study by Labban and Etnier (2011) tested the effect of acute exercise on long-term memory by presenting subjects with two paragraphs after a bout of cycling exercise and reported that these subjects recalled significantly more than non-exercise controls after a 35-minute delay. The subjects that performed exercise *after* the learning phase of the memory task did not recall more than non-exercise controls, demonstrating the benefit of exercise prior to learning. In contrast, exercise following learning has also been shown to be effective at enhancing memory in certain populations (Segal *et al.*, 2012). Segal et al. (2012) showed that an acute bout of exercise after picture viewing enhanced memory for emotional images in older adults with or without mild cognitive impairment. These studies on the benefits of acute exercise on memory are supported by a more recent meta-analysis by Roig et al. (2013), which reported that acute cardiovascular exercise is associated with small to moderate effects on memory with the larger effects (moderate to large) observed for long-term memory (compared to short-term or

working memory). More research is certainly needed to understand the effectiveness of acute exercise on cognitive function. Factors such as intensity of exercise (Winter *et al.*, 2007) might play a role and certain populations might benefit more from acute exercise. For example, populations at risk or suffering from mental health disorders, which may be associated with compromised BDNF levels and other important signaling factors, may be particularly sensitive to properly prescribed acute exercise.

Acute Exercise Increases Peripheral BDNF in Humans

The mechanisms mediating the beneficial effects of acute exercise on memory performance in humans are not fully understood. Based on the findings of chronic exercise training studies in rodents and humans, researchers have attempted to use correlational analyses to support the hypothesis that elevated peripheral BDNF is the mechanism mediating the cognitive enhancement afforded by acute exercise (Piepmeier & Etnier, 2015). Indeed, acute exercise increases peripheral BDNF levels in humans (Vega *et al.*, 2006; Ferris *et al.*, 2007; Winter *et al.*, 2007; Rasmussen *et al.*, 2009; Griffin *et al.*, 2011) and in some studies, the increases are associated with improved cognitive task performance (Ferris *et al.*, 2007; Winter *et al.*, 2007; Griffin *et al.*, 2011). For example, Ferris et al. (2007) showed that serum BDNF was elevated after an acute bout of exercise at an intensity above the ventilatory threshold, but this effect did not persist if the acute exercise was performed at an intensity below the ventilatory threshold. The high-intensity acute exercise was associated with

better performance on the Stroop Color and Word Test, a task of executive functioning. Winter et al. (2007) reported that faster vocabulary learning after high-intensity running was associated with increased levels of BDNF. It is important to recognize that exercise increases levels of many circulating factors and using correlational analyses with selected factors like BDNF should be interpreted with caution. It is difficult to link the elevation of peripheral BDNF levels with improved cognitive task performance since 70-80% of circulating BDNF is released from the brain into systemic circulation and therefore is no longer in the vicinity of the brain regions associated with enhanced cognitive performance (Rasmussen *et al.*, 2009). More studies and a more thorough understanding of the benefits of elevated peripheral BDNF levels for brain health are necessary. Animal models provide an effective way to understand how acute physical activity influences expression of plasticity-associated genes in the hippocampus.

Acute Exercise and Hippocampal Bdnf in Rodents

How a single bout of acute exercise influences hippocampal Bdnf levels is not fully understood. Obstacles to interpreting the acute exercise and hippocampal Bdnf literature include differences in exercise exposures and various acclimation protocols that might induce adaptations independent of the acute exercise or may interact with the acute bout of exercise. Goekint et al. (2012) found no effect of 60 minutes of treadmill running on Bdnf protein in the rat hippocampus immediately following or two hours after an exercise bout.

However, Oliff et al. (1998) reported that both six hours and 12 hours of voluntary wheel running increased *Bdnf* mRNA in the rat hippocampus. Importantly, in this study the rats were housed in cages with voluntary running wheels for three nights to acclimate the rats to the environment. This "training" period was followed by 10 days of no wheel exposure before the acute bout of exercise (six or 12 hours of housing with a running wheel). The authors reported that six hours of running significantly increased *Bdnf* mRNA in CA1, CA3, and the hilus region of the hippocampus. The six and 12 hour runs also significantly increased *Bdnf* exon I expression in the dentate gyrus, CA3, and hilus. Exon II expression was increased in CA1 after 12 hours of running. Exon IV (exon III in the paper) was not elevated following acute exercise; however, this finding is difficult to interpret because researchers reported that rats that underwent three days of "training" followed by 10 days of no-wheel exposure had elevated *Bdnf* mRNA in CA1 and elevated *BDNF IV* in all hippocampal regions examined. This finding is important because it highlights the need to examine truly acute bouts of exercise since even 10 days of sedentary conditions may not negate the effects of previous physical activity. Rasmussen et al. (2009) reported that two hours of treadmill running to exhaustion increased *Bdnf* mRNA in the hippocampus and cortex at two hours post-running but not immediately post-running. Similar to Goekint et al. (2012) and Oliff et al. (1998), the mice in the Rasmussen et al. (2009) investigation underwent a familiarization protocol that involved exercise training followed by a "wash out" prior to the acute bout of exercise. These studies suggest that Bdnf expression can be induced by acute bouts of exercise

but also highlight the need to understand the adaptations to truly acute bouts of exercise. The evidence for rapid induction of Bdnf with other environmental stimuli, such as immobilization stress (Marmigère *et al.*, 2003) suggests that exercise may also be able to induce rapid increases in Bdnf expression. Elevated *Bdnf* mRNA expression following acute exercise may prime the hippocampus for learning by providing the mRNA to be translated during encoding and consolidation and signal for architectural adaptations necessary for the formation and persistence of memory.

Research in humans and rodents demonstrates that acute exercise can influence central and peripheral BDNF levels, though many important questions still remain. For example, what is the importance of peripheral BDNF and what exercise-induced mechanisms are stimulating the production and release of central BDNF? Potentially, circulating factors released in response to exercise activate signaling pathways to increase expression of BDNF. Catecholamines are a promising target as they are influenced by acute exercise, strongly influence memory and plasticity, and regulate BDNF expression.

Catecholamines and Exercise – The Link Between the Periphery and Improved Memory?

Research in stress and emotion has identified arousal and subsequent activation of the noradrenergic (NA) system as a critical and potent memory enhancer (McGaugh, 2013). Both peripheral levels of the catecholamine epinephrine and central levels of the neurotransmitter norepinephrine (which are

strongly correlated and discussed below) are associated with strength of memories. Flashbulb memories, which are very vivid and long-lasting memories of significant and arousing public events (Brown & Kulik, 1977), and PTSD are good examples of the effect of emotion and adrenergic enhancement of memory. Remarkably, both the β-adrenergic receptor blocker propranolol and morphine (which blocks norepinephrine release) decrease the risk of developing PTSD when provided shortly after a traumatic event (Vaiva *et al.*, 2003; Holbrook *et al.*, 2010). Moreover, compromised memory with β-blockers has also been reported in human investigations beyond those concerning PTSD (Chamberlain & Robbins, 2013). For example, using propranolol, Cahill et al. (1994) reported that β-adrenergic receptors were necessary for enhanced memory of emotionally charged stories relative to neutral stories. In humans, both direct epinephrine infusions (Cahill & Alkire, 2003) and cold pressor stress (Cahill, 2003), a treatment that stimulates the release of peripheral epinephrine, improve memory. Segal and Cahill (2009) found a significant correlation between levels of salivary α-amylase (a marker of central NA activation) after viewing a series of images of varying emotional grade and memory recall of those emotional images one week later. In rodents, β-adrenergic receptor blockers, such as propranolol, have been shown to block spatial and contextual fear memories when infused into the hippocampus (Ji, Wang, *et al.*, 2003; Ji, Zhang, *et al.*, 2003), while infusion of norepinephrine into the hippocampus and peripheral injections of epinephrine improve inhibitory avoidance and contextual fear memory, respectively (Izquierdo *et al.*, 1998; Hu *et al.*, 2007). It is beyond the scope of this review to

comprehensively review all of the research on the memory enhancing effects of NA stimulation. Investigations of the effects of NA stimulation on encoding, consolidation, and retrieval of memories spans decades and numerous excellent reviews have been written on the topic (for examples see McGaugh & Roozendaal, 2002; Kensinger, 2009; Chamberlain & Robbins, 2013; McGaugh, 2013; O'Dell *et al.*, 2015; Osborne *et al.*, 2015). Though much research has investigated the memory-enhancing effects of stress and catecholamines, more research is needed to provide useful strategies to utilize the memory-enhancing effects of stress and catecholamines. Potentially, exercise can be used as a non-invasive and non-traumatic environmental stimulus to activate the NA system to enhance memories similar to the enhanced memory observed with psychological stress.

Circulating epinephrine might be the signaling factor between the periphery and the hippocampus during exercise that mediates the enhancement of memory and hippocampal plasticity. In a 2002 review of literature on acute bouts of physical activity and cognitive performance, it was suggested that for an acute bout of exercise to impact cognition, the exercise must be intense enough to increase circulating catecholamines (Brisswalter *et al.*, 2002). Indeed, exercise of sufficient duration and intensity increases peripheral catecholamines, a necessary response for cardiovascular and metabolic adjustments to the acute exercise (Tipton, 2006; Zouhal *et al.*, 2008). Exercise of short duration and high intensity, or long duration and low intensity is sufficient to significantly elevate peripheral levels of catecholamines (Zouhal *et al.*, 2008). This is due to an

increase in catecholamine secretion and potentially a reduction in catecholamine clearance (Zouhal *et al.*, 2008). Previous research has tried to identify if exercise-induced arousal improves cognition, though many studies have employed cognitive tests that measure simple cognitive domains such as reaction time and employed the cognitive task during exercise (McMorris *et al.*, 2008; 2009). Potentially, the cognitive resources required during some types of exercise (e.g. treadmill exercise) may impair performance during exercise (Lambourne & Tomporowski, 2010) and more research is needed specifically examining memory, which is not comparable to tasks assessing reaction time. However, there is evidence of a relationship between acute exercise-induced elevations of epinephrine and memory performance (Winter *et al.*, 2007). An investigation by Winter et al. (2007) reported that high-intensity running increased peripheral levels of epinephrine and memory performance on a word recall task at one week and eight months after learning. The improved memory performance was positively correlated with epinephrine levels. Though epinephrine does not directly influence hippocampal plasticity and improve memory, peripheral epinephrine has direct influences on central levels of the plasticity-promoting neurotransmitter, norepinephrine.

Peripheral Epinephrine Increases Central Norepinephrine Release

Unlike cortisol and other circulating factors, epinephrine does not cross the blood-brain barrier. Instead, epinephrine mediates its effects through activation of β-adrenergic receptors on vagal afferents that terminate on brainstem NA cell

groups in the nucleus of the solitary tract (NTS) (McGaugh & Roozendaal, 2002). The NTS stimulates release of norepinephrine from the locus coeruleus (LC), which has extensive innervations to numerous brain regions, including the hippocampus (Osborne *et al.*, 2015). Miyashita and Williams (2004) demonstrated that an intraperitoneal (IP) injection of saline had no effect on hippocampal extracellular levels of norepinephrine, however following a single IP injection of epinephrine there was a significant increase in hippocampal norepinephrine. This finding demonstrates that a stimulus that increases peripheral levels of epinephrine (e.g. acute exercise) can increase hippocampal levels of norepinephrine. Remarkably, stimulation of the ascending vagus nerve (which increases central norepinephrine) post-learning improves memory (Clark *et al.*, 1998), consistent with the effects of epinephrine infusions (McGaugh, 2013). In addition, blocking NTS activity with lidocaine blocks the effects of peripheral epinephrine on memory consolidation (Williams & McGaugh, 1993). These investigations demonstrate that peripheral epinephrine stimulates the ascending vagus nerve-LC-NA system and exerts its memory enhancement by increasing levels of central norepinephrine. It is important to note that glucocorticoids are also known to be important for the memory enhancing effects of acute psychological stress (McGaugh & Roozendaal, 2002) but it is likely that glucocorticoids and catecholamines work in concert to influence memory and plasticity (Osborne *et al.*, 2015). In fact, blocking NA signaling prevents the memory enhancing effects of glucocorticoids (Quirarte & Roozendaal, 1997; Roozendaal & Nguyen, 1999). Roozendaal and Nguyen (1999) blocked NA

signaling in the amygdala using the β-adrenergic blocker atenolol and attenuated the memory-enhancing effects of direct infusions of a glucocorticoid agonist into the dorsal hippocampus.

Central Norepinephrine Levels and Exercise

In addition to an increase in peripheral catecholamines, rodent research suggests that central norepinephrine levels are elevated during exercise (Pagliari & Peyrin, 1995a; 1995b). Pagliari et al. (1995a) found that acute treadmill exercise increased circulating epinephrine, brain norepinephrine, and the norepinephrine metabolite MHPG in an exercise duration-dependent manner in the rat cortex. They observed that the elevation in peripheral epinephrine preceded the increase in central norepinephrine, which is consistent with the idea that exercise-induced release of epinephrine stimulates the exercise-stimulated release of central norepinephrine. However, the literature reporting an effect of acute exercise on hippocampal levels of norepinephrine are not conclusive. Goekint et al. (2012) found no effect of one hour of treadmill running on hippocampal norepinephrine in rats, using microanalysis during and after the running. Dunn et al. (1996) reported that chronic treadmill running increased basal hippocampal levels of the extraneuronal norepinephrine metabolite MHPG, suggesting release and subsequent breakdown of norepinephrine, but no effect on hippocampal norepinephrine levels. Interpretation of research measuring norepinephrine levels in the hippocampus following exercise is difficult, as it is not clear which measurement technique (extra-synaptic, tissue content,

metabolites) most accurately reflects biologically relevant norepinephrine in the hippocampus. Research in humans supports that acute exercise increases central noradrenergic activation. Measuring salivary α-amylase is an effective way to predict central NA activation (Chatterton *et al.*, 1996) and research shows that levels of salivary α-amylase increase with exercise (Chatterton *et al.*, 1996; Allgrove *et al.*, 2008; Segal *et al.*, 2012). Segal et al. (2012) reported that exercise-induced elevation in salivary α-amylase was correlated with performance on a memory recall trial. These researchers had older adults view a series of images of varying emotional charge followed by a six-minute bout of exercise at 70% VO_2max, which was sufficient to increase salivary α-amylase and improve memory for emotional images.

Norepinephrine and Plasticity

Norepinephrine is important for normal brain function and a potent memory enhancer. In addition to activation of the ERK pathway (O'Dell *et al.*, 2015) and influencing glucose metabolism (Osborne *et al.*, 2015) in the brain, norepinephrine induces plasticity through glutamate receptor phosphorylation and CREB-mediated transcription. Norepinephrine is a NA neurotransmitter that is synthesized and released from the LC. The LC projects to numerous brain regions including the amygdala, frontal cortex, and hippocampus. The β-adrenergic receptor is a G-coupled protein receptor that induces numerous downstream signaling cascades (through cyclic AMP) including posttranslational modifications to key plasticity-mediating molecules and CREB-mediated

transcription (Gelinas, 2005; McGaugh, 2013). Glutamate is the major neurotransmitter of the central nervous system (CNS), and glutamate receptors (AMPA, NMDA, mGluR) mediate the majority of synaptic communication in the mammalian brain. The AMPA type glutamate receptor consists of multiple GluR subunits (GluR1-4) and is critical for synaptic communication and plasticity (Santos *et al.*, 2009). The adult mammalian brain contains heterotetramers composed of GluR1/GluR2 and GluR2/GluR3 combinations (Santos *et al.*, 2009). Phosphorylation of specific sites (Ser831 and Ser845) on GluR1 is critical for the synaptic activity-induced extra-synaptic insertion of the receptor and subsequent migration to the synapse (Huganir & Nicoll, 2013). Extra-synaptic insertion lowers the threshold for LTP, a cellular representation of learning and memory (Oh *et al.*, 2006). Hu et al. (2007) showed that norepinephrine exposure to hippocampal neurons in culture increased phosphorylation of Ser845 and Ser831 and reduced the threshold for LTP (Hu *et al.*, 2007). IP injections of epinephrine in the mouse increased phosphorylation of Ser845 (the PKA dependent site; downstream of β_2-adrenergic receptor) on GluR1 and reduced the threshold for learning (Hu *et al.*, 2007). The effect of norepinephrine on AMPA receptor phosphorylation is dependent on the β-adrenergic receptor, as exposure to fox urine induced Ser845 phosphorylation, but this effect was blocked by pretreatment with the β-adrenergic receptor blocker propranolol (Hu *et al.*, 2007). Importantly, when the authors used a mutant mouse with a knock-in mutation that prevented phosphorylation of Ser845 and Ser831, IP injections of epinephrine no longer reduced the threshold for learning. An investigation by

Chai et al. (2014) showed that the enhancement of extinction memory afforded by infusions of norepinephrine into the dorsal hippocampus was associated with increased membrane GluR1, Ser845 phosphorylation, and CREB phosphorylation. Enhanced learning/memory and a lower threshold for LTP and learning have also been observed following short-term exercise training (Farmer *et al.*, 2004; Intlekofer *et al.*, 2013). Understanding the mechanisms and stimuli that cause phosphorylation and membrane insertion of the GluR1 subunit is a focus of intense investigation and exercise-induced norepinephrine release is a potential mechanism to induce this favorable adaptation. Moreover, β-adrenergic signaling plays a crucial role in acute physical-stress induced transcription in the rodent hippocampus (Roszkowski *et al.*, 2016) and might be a critical factor in exercise induced-Bdnf expression.

Noradrenergic System and Exercise Training-Induced Plasticity and Bdnf Expression

The beneficial adaptations in the hippocampus following exercise training are blocked by peripheral administration of propranolol (Ivy *et al.*, 2003) or DSP-4 (Garcia *et al.*, 2003), a LC-NA-selective neurotoxin. Based on the similarities between the effects of antidepressant treatment and physical activity on the hippocampus (e.g. increased Bdnf, neurogenesis), researchers suggested that, like antidepressants, which increase and prolong extrasynaptic monoamine levels, exercise might mediate its effects through elevated norepinephrine. Ivy et al. (2003) demonstrated that three days of housing with a voluntary running

wheel increased hippocampal *Bdnf* mRNA levels but this was blocked by nightly IP injections of propranolol over those three days. Garcia et al. (2003) reported that one week of voluntary wheel exposure increased total *Bdnf* mRNA expression in the rat hippocampus and this was attenuated in rats pre-treated with DSP-4 one week prior to wheel exposure. The researchers also investigated the effect of exercise and DSP-4 on exon-specific *Bdnf* transcription. Exon I expression was elevated with one week of exercise in the dentate gyrus and DSP-4 significantly attenuated this. Exon II expression was actually increased in CA1, CA2, and CA3 with combined exercise and DSP-4. In fact, exercise mice injected with saline had significantly less exon II expression compared to exercise mice injected with DSP-4 and there was no effect of exercise alone on exon II mRNA expression. Similar results were observed for exon IV (exon III in the paper), with DSP-4 actually increasing levels of exon IV expression and no effect of exercise alone. The discrepancy between total *Bdnf* mRNA and transcript-specific expression is curious and deserves more research. This could be due to the unusual properties of DSP-4, which lesions NA nerve terminals and drastically reduces tissue content of norepinephrine in the hippocampus, but has also been shown to increase levels of extrasynaptic norepinephrine (Ross & Stenfors, 2014). To support the effect of norepinephrine on Bdnf, Chen et al. (2007) showed that norepinephrine increased Bdnf protein expression and signaling in hippocampal culture. Baj et al. (2012) reported that incubating neurons with norepinephrine increases *Bdnf* mRNA targeting to the dendrites and this was mediated primarily by *Bdnf* exon VI. These data

demonstrate an important relationship between exercise, the NA system, and Bdnf, however the relationship between acute exercise and central NA-mediated enhancement of hippocampal plasticity and Bdnf expression is unknown.

Acute Exercise and Anxiety

Norepinephrine is strongly associated with the pathogenesis of anxiety. Interestingly, norepinephrine is associated with both anxiolytic and anxiogenic behavioral responses (Goddard *et al.*, 2010). In response to acute stress, NA neurons in the LC and/or signals from the limbic system stimulate the HPA axis, resulting in release of corticotropin-releasing hormone from the paraventricular nucleus of the hypothalamus (PVN), which then stimulates the production and secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland. Upon release of ACTH, corticosterone is released from the adrenal cortex (Goddard *et al.*, 2010). In addition to the aforementioned HPA axis, stress signals arising from the limbic system or hypothalamus stimulate the LC to release norepinephrine centrally and further stimulates the release of norepinephrine from the sympathetic nervous system and norepinephrine and epinephrine from the adrenal medulla (Sothmann *et al.*, 1996). These adaptations to stress – whether psychological or physical – are important for the "fight or flight" response , though in rodents, avoidance in the form of freezing is a common response to stressful situations (Goddard *et al.*, 2010) and is frequently used as a measure of anxiety and fear in rodent behavioral paradigms. Though high levels of catecholamines can result in strong memory enhancement, as seen with

flashbulb memories, it is reasonable to assume that high-intensity exercise that increases catecholamine levels may induce anxiety and be detrimental to cognitive task performance. Numerous investigations have been conducted to determine the influence of chronic exercise on anxiety in humans and anxiety-like behavior in rodents, but the results have been inconclusive. In rodents, some investigations report anxiolytic effects while others report anxiogenic effects of exercise (Sciolino & Holmes, 2012). The effects of acute exercise and exercise-induced stress hormones on anxiety-like behavior are not understood, especially in animal models where the effects of exercise on anxiety have been almost exclusively studied in the context of chronic exercise training and response to stressful situations.

Two meta-analyses, one examining research pre-1991 (Petruzzello *et al.*, 1991) and one examining research since 1991 (Ensari *et al.*, 2015) have been conducted and support that acute exercise has small but significant beneficial effects on state anxiety in humans. However, exercise that exceeds the anaerobic or ventilatory threshold has been associated with reduced affect during exercise (Bixby *et al.*, 2001; Ekkekakis *et al.*, 2008; Lind *et al.*, 2008) and other research provides evidence of increased feelings of stress after acute exercise (Hopkins *et al.*, 2012). Even though exercise in humans rarely results in high levels of anxiety or increased rates of panic attacks (O'connor *et al.*, 2000), the reduced affect observed in humans may present itself as increased anxiety in rodents since the acute high-intensity exercise is uncontrollable, novel, and results in increased stress hormone levels. Duman et al. (2008) reported

increased anxiety-like behaviors following three weeks of voluntary wheel running if the behavioral task took place the morning after a night of voluntary running wheel access. If the task was performed 24 hours after cessation of voluntary running, the animal displayed reduced anxiety-like behavior. Other studies have also reported anxiogenic effects of chronic voluntary exercise in rodents (Fuss *et al.*, 2009; 2010; Onksen *et al.*, 2012) and Dishman et al. (1996) reported that eight weeks of treadmill running reduced activity in the open field task (anxiety-like behavior) 24 hours after the last running exposure but eight weeks of voluntary wheel running increased activity in the open field task (anxiolytic-like behavior).

The influence of acute exercise on animal behavior is important to understand since animal models are commonly used to examine the effectiveness of treatment strategies. Acute exercise is a promising approach to treat conditions such as PTSD (Powers *et al.*, 2015), but a potential anxiety-like effect observed in animals might interfere with the interpretation of mechanistic and behavioral findings in rodents. For this reason, it is important to fully characterize the behavioral response to acute exercise in animal models to determine the most effective way to use these models to understand the application of acute exercise for therapies and cognitive training.

Training Influences Catecholamine Response to Acute Exercise

Exercise training in humans is associated with a reduced catecholamine response to the same absolute exercise intensity but a higher capacity to

increase catecholamine release at maximal exercise intensities (Kjaer, 1992; Zouhal *et al.*, 2008). Similarly, in rodents, exercise training is associated with an increased capacity for peripheral epinephrine release (Zouhal *et al.*, 2008); however, the available literature in rodents suggests that previous exposure to exercise reduces the central NA response to both homotypic (e.g. exercise) and heterotypic physical or psychological stressors (e.g. immobilization stress). Concerning running, Pagliari and Peyrin (1995b) reported that mice trained for 12 days to run for one hour had significantly increased cortical norepinephrine during a two hour run compared to mice trained for 12 days to run for two hours. Dishman et al. (1997) reported that rats housed with a voluntary running wheel for 9-12 weeks before exposure to uncontrollable foot shock and an escape shuttle box task had 61% higher levels of norepinephrine in the LC compared to sedentary controls. In mice that experienced controllable footshock, LC norepinephrine concentrations were 49% higher than sedentary controls. These data indicate that wheel running protects against depletion of norepinephrine in the LC. The protection of LC- norepinephrine depletion with exercise was also confirmed in an investigation that utilized six weeks of forced treadmill running prior to exposure to immobilization stress or treadmill running (Dishman *et al.*, 2000). The blunting of norepinephrine depletion with stress was observed in the LC and brain regions innervated by the LC including the amygdala and the hippocampus. After 40 minutes of a scrambled footshock, rats housed with a voluntary running wheel for 4-5 weeks had significantly lower levels of norepinephrine in the frontal cortex compared to rats housed in standard cages

(Soares *et al.*, 1999). The authors suggested this demonstrated a reduction in the stress-induced release of norepinephrine following housing with a running wheel. Not only is norepinephrine release and depletion influenced by voluntary wheel and forced treadmill running but stress-induced activity in the LC is also reduced. Rats housed with running wheels for six weeks have significantly reduced c-Fos expression in the LC following inescapable tail shocks compared to mice housed with locked wheels (Greenwood *et al.*, 2003). Taken together, these studies provide strong evidence that exercise training reduces the stress response in rodents to both homotypic and heterotypic stressors. Knowing that norepinephrine and β-adrenergic signaling (Garcia *et al.*, 2003; Ivy *et al.*, 2003) are important for Bdnf expression, this reduction in norepinephrine release following exercise training provides the potential for a reduction in acute exercise-induced Bdnf expression following exercise training.

Training Influences Bdnf Response to Acute Exercise

Cardiovascular fitness or a previous history of physical activity influences the peripheral BDNF response to acute exercise (Knaepen *et al.*, 2010). Griffin et al. (2011) reported that an acute bout of exercise to exhaustion increased serum BDNF immediately after the exercise bout but this was attenuated in subjects who underwent three or five weeks of aerobic exercise training. Wagner et al. (2015) reported a similar blunting of the peripheral BDNF response to exhaustive acute exercise following eight weeks of exercise training. It is important to note that the acute exercise protocols in both studies were to

exhaustion before and after training, so the effects of the acute exercise cannot be attributed to reduced relative exercise intensity. Not all studies have reported a blunting of acute exercise-induced BDNF response, however. Seifert et al. (2010) reported an increase in BDNF release from the human brain (brachial artery – jugular vein difference) with acute exercise of varying intensities and reported no effect of three months of aerobic training on acute exercise stimulated release.

In contrast to what has been observed peripherally in humans, research in the rodent hippocampus suggests that previous exposure to exercise increases the ability to increase Bdnf levels with an additional exposure to exercise. Berchtold et al. (2005) exposed rats to a voluntary running wheel either daily or every other day over a 28 day period; both of these protocols increased Bdnf protein in the hippocampus. After a period of seven or 14 days of no wheel running, two days of voluntary wheel running increased Bdnf expression in the hippocampus only in mice that were previously exposed to voluntary wheel running. An interesting finding from this investigation was that even though intermittent running every other day was as effective at increasing Bdnf in the hippocampus as daily running, Bdnf protein returned to baseline by day three of sedentary conditions in the intermittent group but remained elevated for a week in the daily running group. This demonstrates that while various exercise exposures may result in similar elevations in Bdnf, the lasting benefits may differ depending on the stimulus. Concerning transcriptional regulation of *Bdnf*, Gomez-Pinilla et al. (2010) reported that one week of voluntary wheel running

reduced methylation and increased mRNA expression of *Bdnf IV*. Reduced methylation of this transcript suggests increased transcriptional capability, though how long this reduced methylation persists is unknown. These studies in rodents suggest that previous exposure to voluntary wheel running augments the Bdnf response to voluntary wheel running. It is not known if previous exposure to voluntary wheel running will augment the Bdnf response to forced treadmill exercise, given that the physical and psychological stress associated with forced treadmill running is different from voluntary wheel running.

Summary

Much research has been done over the past 30 years to understand how exercise influences the human and rodent brain. Though much has been discovered, many questions remain unanswered. Only with rigorous investigations utilizing animal models and unique exercise approaches can exploration into the mechanisms mediating the favorable hippocampal adaptations to acute and chronic exercise be truly accomplished. Though animal models are currently necessary for this, careful consideration must be taken when translating exercise and behavioral observations to human application. This dissertation project contains a series of investigations designed to address many of the questions presented in the review of literature and provides exciting new information on the influence of both acute and chronic exercise on markers of hippocampal plasticity and animal behavior.

Chapter 3.

Aim #1: Determine the effect of five months of voluntary wheel exposure on hippocampal mRNA expression of plasticity-associated genes in adult male and female mice.

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Title: Sex-Dependent and Independent Effects of Long-Term Voluntary Wheel Running on Bdnf mRNA and Protein Expression

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Abstract

The beneficial effects of physical activity on brain health (synaptogenesis, neurogenesis, enhanced synaptic plasticity, improved learning and memory) appear to be mediated through changes in region-specific expression of neurotrophins, transcription factors, and postsynaptic receptors, though investigations of sex differences in response to long-term voluntary wheel running are limited. **Purpose:** To examine the effect of five months of voluntary wheel running on hippocampal mRNA and protein expression of factors critical for exercise-induced structural and functional plasticity in male and female adult mice. **Methods**: At 8 weeks of age, male and female C57BL/6 mice were individually housed with (PA; n=20; 10 male) or without (SED; n=20; 10 male) access to a computer monitored voluntary running wheel. At 28 weeks, all mice were sacrificed and hippocampi removed. Total RNA was isolated from the hippocampus and expression of total *Bdnf, Bdnf* transcript IV, *tPA*, *Pgc-1a*, *GluR1*, *NR2A*, and *NR2B* were assessed with quantitative RT-PCR and total and mature Bdnf protein were assessed with ELISA. **Results:** We found significantly higher *Bdnf IV* mRNA expression in PA males (p=0.03) and females (p=0.03) compared to SED animals. Total *Bdnf* mRNA expression was significantly greater in PA males compared to SED males (p=0.01), but there was no difference in females. Similarly, we observed significantly higher mature Bdnf protein in PA males compared to SED males (p=0.04), but not in females. **Conclusion:** These findings indicate that the impact of long-term voluntary wheel running on transcriptional and post-translational regulation of Bdnf may be sex-

dependent, though the activity-dependent *Bdnf IV* transcript is sensitive to exercise independent of sex.

Key Words: Brain-Derived Neurotrophic Factor; Sex-Differences; Exercise; Physical Activity; Hippocampus

Introduction

Chronic exercise training and physical activity have remarkable effects on the human and rodent hippocampus (Voss *et al.*, 2013). Structural adaptations observed in the hippocampus in response to exercise training and physical activity include synaptogenesis, dendritic arborization, and neurogenesis (Eadie *et al.*, 2005; Redila & Christie, 2006; Stranahan *et al.*, 2007; Lin *et al.*, 2012) while functional adaptations include enhanced learning and memory, increased amplitude of long-term potentiation (LTP), and reduced threshold for LTP (van Praag, Christie, *et al.*, 1999; Kida *et al.*, 2002; Farmer *et al.*, 2004; Titterness *et al.*, 2011). Moreover, hippocampal neurons show increased mitochondrial biogenesis in response to chronic exercise training (Steiner *et al.*, 2011). These structural and functional adaptations are believed to be the result of increased expression of important neurotrophins, transcription factors, and postsynaptic receptors.

Elevated expression (mRNA and/or protein) and downstream signaling of brain-derived neurotrophic factor (BDNF) has been identified as a primary exercise-induced regulator of functional and structural plasticity since blocking Bdnf expression or signaling attenuates improvement in learning, memory, and expression of genes important for synaptic plasticity in the hippocampus following exercise training (Vaynman *et al.*, 2004; Gomez-Pinilla *et al.*, 2008). Circulating BDNF is elevated in humans following acute exercise and exercise training (Gold *et al.*, 2003; Vega *et al.*, 2006; Ferris *et al.*, 2007; Erickson *et al.*, 2011). In the rodent hippocampus, Bdnf protein and mRNA are elevated

following brief exercise exposures (≤ 7 days) (Neeper *et al.*, 1995; 1996; Molteni *et al.*, 2002; Vaynman *et al.*, 2003; Berchtold *et al.*, 2005; Huang *et al.*, 2005; Ding *et al.*, 2011; Sartori *et al.*, 2011) and longer exercise exposures (>7 days) (Molteni *et al.*, 2002; Farmer *et al.*, 2004; Berchtold *et al.*, 2005; Liu *et al.*, 2009; Berchtold *et al.*, 2010; Ding *et al.*, 2011; Kobilo *et al.*, 2011; Sartori *et al.*, 2011; Marlatt *et al.*, 2012; Wrann *et al.*, 2013; Darlington *et al.*, 2014). Importantly, most studies in rodents that attempt to address mechanisms mediating the cognitive enhancing effects of chronic exercise have focused on exercise training ranging from ~7 days to 3 months and how longer voluntary wheel exposures influence mRNA expression of plasticity-associated genes is not fully understood. When mice are exposed to a voluntary running wheel, activity decreases over time (Richter *et al.*, 2014; Venezia *et al.*, 2015) and focusing on short-term chronic exercise favors plasticity by highlighting the response to the high wheel activity and the novelty of activity. This might present a biased view of the benefits of chronic exercise training on plasticity-associated gene and protein expression. However, Marlatt et al. (2012) showed that eight months of voluntary wheel running increased Bdnf protein expression in 17-month old female C57BL/6J mice that began running at nine months of age. This suggests that long-term voluntary exercise maintains elevated Bdnf expression that is normally observed following short-term exercise exposure. It is not fully understood how long-term voluntary wheel exposure influences young-adult male and female hippocampal plasticity-associated gene and protein expression.

Importantly, the *Bdnf* gene is highly complex, containing eight non-coding exons with individual promoters that all splice to one 3' protein coding exon (exon IX). Though *Bdnf* is considered an activity-regulated gene, promoter IV-driven *Bdnf* transcription is especially sensitive to neuronal activity (Tao *et al.*, 1998; 2002) and environmental stimuli (Lubin *et al.*, 2008; Intlekofer *et al.*, 2013). Interestingly, all *Bdnf* transcripts are translated into the same protein (proBDNF), which is then cleaved to produce the mature plasticity-associated protein. It is not fully understood how long-term voluntary exercise influences the transcription and post-translational processing of Bdnf.

The hippocampus is a sexually dimorphic structure (Madeira & Lieberman, 1995) and environmental stimuli result in sex-dependent hippocampal adaptations (Cahill, 2006). In fact, many stimuli will result in similar behavioral responses in males and females, but the mechanisms by which these responses are mediated may be different between the sexes (Cahill, 2006). Exercise results in beneficial adaptations to the hippocampus in both males (Farmer *et al.*, 2004) and females (van Praag, Christie, *et al.*, 1999), though it is not known if exercise is stimulating the same signaling pathways in both sexes. Further, research in adolescent rats supports that sex differences exist in the hippocampal response to exercise (Titterness *et al.*, 2011). The purpose of the present investigation was to examine how five months of voluntary wheel running influences hippocampal mRNA and protein expression in adult male and female C57BL/6J mice. We hypothesized that long-term chronic voluntary wheel running would have small or no effects on hippocampal mRNA expression of

plasticity-associated genes due to reduced wheel running over time and that any observed differences in mRNA expression would be sex-dependent. We focused our investigation on Bdnf mRNA and protein as well as other genes important for the effect of exercise on structural and functional plasticity, mitochondrial biogenesis, and synaptic transmission.

Methods

Animals and Voluntary Wheel Running: Male and female C57BL/6J mice were used in this investigation. All animals were cared for by University of Maryland veterinary staff and kept on 12hr light/12hr dark cycle and provided standard rodent chow *ad libitum*. All protocols were IACUC approved. At eight weeks of age, male and female C57Bl/6J mice were individually housed with (n=20; 10 male) or without (n=20; 10 male) continuous access to a computer-monitored voluntary running wheel (Lafayette Instruments, Lafayette IN). Mice were sacrificed at 28 weeks of age.

Tissue Collection & Processing: All mice were exposed to intraperitoneal glucose tolerance testing (IPGTT) 24 hours before sacrifice. Mice were fasted (ad libitum water access) for 6 hours prior to IPGTT. Baseline blood glucose measurements were made and then each mouse was injected intraperitoneally with 2.0 mg of D-glucose (Sigma-Aldrich, St. Louis, MO) per gram of body mass. Blood glucose was measured 15, 30, 60, 90, and 120 minutes after injection in all animals. All blood glucose measurements were made on blood removed from a single tail snip. Following the glucose tolerance test animals were returned to ad

libitum food and water access. On the day of sacrifice, total body mass of anesthetized mice was recorded and mice underwent euthanasia by exsanguination followed by removal of the heart under isoflurane anesthesia. The hippocampus was isolated, halved, and immediately frozen in liquid nitrogen.

Gene Expression: Prior to nucleic acid isolation, hippocampi were homogenized in TRIzol reagent (Life Technologies, Grand Island, NY, USA) using a glass Dounce homogenizer. Total RNA was isolated with TRIzol reagent following manufacturers instructions and quantified via spectrophotometry. Reverse transcription was performed with 1 µg of total RNA with the High-Capacity cDNA RT kit (Life Technologies). Real-time quantitative PCR (qPCR) was used to assess mRNA expression of total *Bdnf* (exon IX); *Bdnf* exon IV (*Bdnf IV*); peroxisome proliferator-activated receptor γ coactivator 1 alpha (*Pgc-1α)*; tissue plasminogen activator (*tPa*); glutamate receptor, ionotropic, AMPA 1 (*GluR1)*; glutamate receptor, ionotropic, NMDA2A (*NR2A)*, glutamate receptor, ionotropic, NMDA2B (*NR2B)*; and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*; expression control; primer sequences listed in Supplemental Table 1). Primer:probe assays were purchased pre-made *(Pgc-1α, tPa, GluR1, NR2A, NR2B, Gapdh)* or designed (*Bdnf IX, Bdnf IV*) for the mRNA sequence of each gene using Integrated DNA Technologies' PrimeTime qPCR Assay designer. All primer pairs except *Bdnf* total spanned exons to prevent amplification of genomic DNA. Because *Bdnf* total is represented by amplification of only exon IX, this primer pair could not span exons. Efficiency for each primer:probe assay was determined prior to use.

Bdnf Protein: Total and mature Bdnf protein levels were measured using the E-Max Bdnf ELISA kit (Promega, WI, USA) according to manufacturer's instructions. Tissues were homogenized on ice in lysis buffer [137mM NaCl, 20mM Tris-HCl (pH 8.0), 1% NP40, 10% glycerol, 0.5mM sodium vanadate, and protease inhibitor cocktail (complete mini EDTA-free protease inhibitors, Roche, 1 tablet/10ml)]. Homogenized samples were diluted in two volumes DPBS containing calcium and magnesium (Life Technologies, NY, USA) and centrifuged for 3 min. at 13,500 rpm at 4^{o}C (Berchtold *et al.*, 2005). Supernatant was collected and total protein concentration determined by a Bicinchoninic acid (BCA) assay following manufacturer's instructions (Pierce Biotechnology, IL, USA). Samples were then diluted in 1x block and sample buffer. For determination of total Bdnf, samples were acidified with 1N HCl for 15 minutes to pH 2-3 and neutralized with 1N NaOH to pH 7-8. The standard curve produced from Bdnf standard dilutions produced an R-value of >0.99.

Statistics: T-tests were used to test for differences in body mass and IPGTT area under the curve (AUC). Running wheel activity was analyzed with a repeated measures ANOVA. Protein and mRNA data were analyzed by two-way ANOVA (exercise x sex) and pre-planned LSD post hoc contrasts to compare exercise vs. sedentary within sexes and male vs. female within exercise conditions. A $p \leq 0.05$ was considered statistically significant.

Results

Wheel Running: Wheel running data are shown in Figure 1. The repeated measures ANOVA revealed a significant effect of time ($F_{(3,12)}$=14.80; p=0.0002) and a tendency for an interaction between sex and time ($F_{(3,12)}$=3.22; p=0.06) for average distance ran per 24 hours. Females ran significantly more during week 1 than males ($t_{(10.6)}$=2.24; p<0.05); however, during week 20, males ran significantly more than females ($t_{(16)}$=-2.94; p<0.01).

Body Mass and GTTs: Female runners weighed significantly less than their sedentary counterparts after five months of running wheel exposure ($t_{(18)}$=-2.37; p=0.03; Fig. 2). There was a tendency for male runners to weigh significantly less than sedentary males ($t_{(17)}$=-1.92; p=0.07; Fig. 2). There was no significant effect of wheel running on blood glucose response (AUC) to an IP injection of glucose (data not shown).

Gene Expression: Gene expression data are shown in Figures 3 and 4. The two-way ANOVA revealed a main effect of exercise ($F_{(1,33)}$=10.89; p=0.002) but no main effect of sex or an exercise by sex interaction on *Bdnf IV* (Fig. 3). Five months of voluntary wheel running led to significantly greater *Bdnf IV* gene expression compared to sedentary living conditions (Fig. 3a) and this effect remained when sexes were separated in the analysis (males: $t_{(16)}$=2.41, p=0.03, Fig. 3b; females: $t_{(17)}$=2.32, p=0.03, Fig. 3c). The two-way ANOVA revealed a significant interaction between exercise and sex for total *Bdnf* mRNA ($F_{(1,33)}$=4.98; p=0.03). There was no main effect of exercise or sex on total *Bdnf*

mRNA levels. Post-hoc analysis revealed that when the sexes were separated, exercise males had significantly higher total *Bdnf* mRNA expression compared to sedentary males ($t_{(16)}$=2.76; p=0.01, Fig. 3b) and this was not observed in females (Fig. 3c). There was no significant effect of five months of voluntary wheel running or sex on *Pgc-1a*, *tPa*, or glutamate receptor subunit expression in either sex (Figs. 3 and 4).

Bdnf Protein: The two-way ANOVA revealed no significant effects of exercise or sex or an interaction between exercise condition and sex on total (Fig. 5) or mature (Fig. 6) Bdnf protein. However, based on our mRNA data, we analyzed by sex and found that mature Bdnf levels were significantly higher in exercise males compared to sedentary males ($t_{(15)}$=2.31, p=0.04, Fig. 6b), an effect not observed in females. Moreover, sedentary males had significantly lower mature Bdnf protein compared to sedentary females ($t_{(16)}$=2.25, p=0.04, Fig. 6b).

Figure 1. Average daily running distance for male and female C57Bl/6J mice. Females ran significantly more per day during week one ($p=0.03$) and significantly less per day during week twenty ($p<0.01$) compared to males. Error bars represent SEM

Figure 2. Male and female body mass. Females with access to voluntary running wheels had significantly lower body mass compared to sedentary females ($p=0.03$). There was a tendency for males with access to voluntary running wheels to have lower body mass compared to sedentary males ($p=0.07$). *$p<0.05$; #$p<0.1$. Error bars represent SEM.

Figure 3. Five months of voluntary wheel running increases Bdnf transcription in a transcript and sex-dependent manner. Target mRNA expression is presented as ddCt relative to *Gapdh*. A) qPCR analysis indicated that five months of wheel exposure increased *Bdnf IV* in the combined sample ($p=0.004$) but did not influence *Bdnf* total, *Pgc1a*, or *tPa* expression. B) Five months of wheel exposure increased *Bdnf IV* ($p=0.03$) and total *Bdnf* ($p=0.01$) expression but had no effect on *Pgc1a* or *tPa* expression in males. C) Five months of wheel exposure increased *Bdnf IV* ($p=0.03$) expression but not total *Bdnf*, *Pgc1a*, or *tPa* in females. Error bars represent SEM.

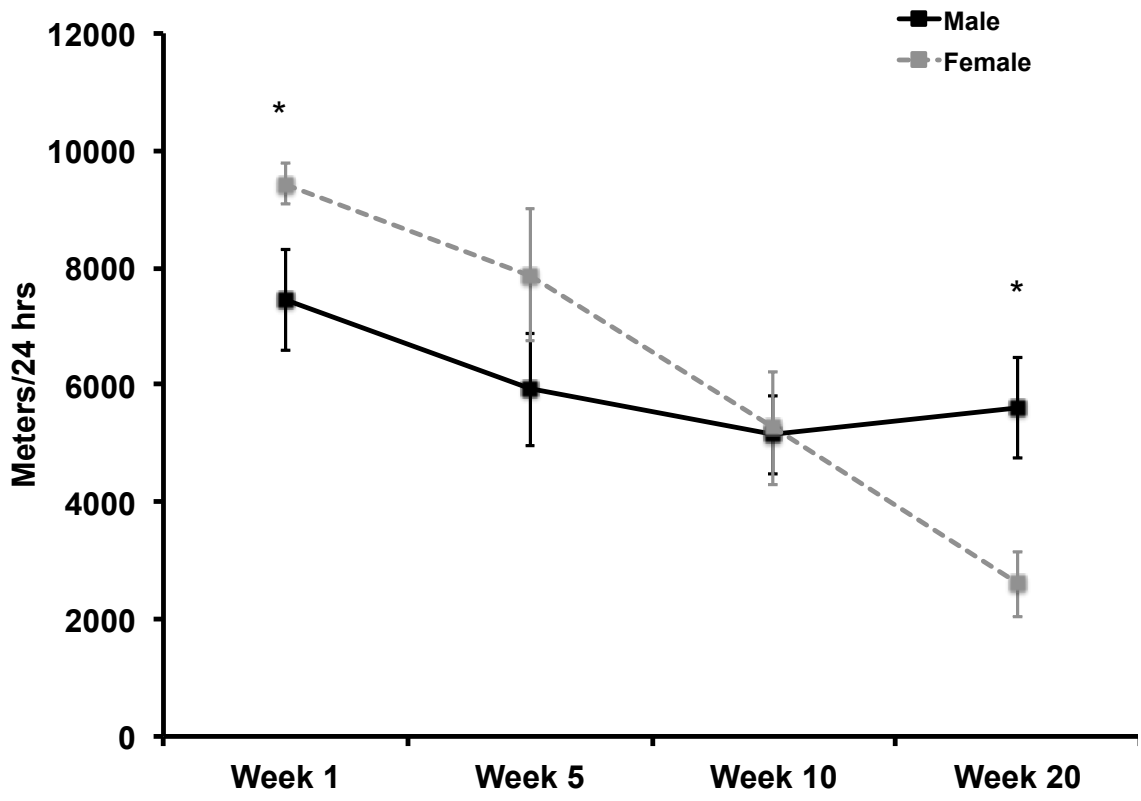
Figure 4. Five months of voluntary wheel running does not influence hippocampal GluR1, NR2A, NR2B, and NR2B/NR2A mRNA expression. Target mRNA expression is presented as ddCt relative to *Gapdh*. qPCR analysis indicated that there was no significant effect of chronic wheel exposure on *GluR1*,

NR2A, or *NR2B* mRNA expression in males, females, or the combined sample. Error bars represent SEM.

Figure 5. Five months of voluntary wheel running does not influence hippocampal total Bdnf protein levels. A) Total Bdnf protein in combined sample of males and females. Data presented as percent of sedentary controls. Five months of wheel exposure did not influence total Bdnf protein expression. B) Five months of wheel exposure did not significantly affect total Bdnf protein expression in males or females. Error bars represent SEM.

Figure 6. Five months of voluntary wheel running significantly increases mature Bdnf protein levels in the male hippocampus. A) Mature Bdnf protein in combined sample of males and females. Data presented as percent of sedentary controls. Five months of wheel exposure did not influence mature Bdnf protein expression. B) Five months of wheel exposure increased mature Bdnf protein expression in males ($p=0.04$) but had no effect in females. Sedentary females had significantly higher mature Bdnf protein expression compared to sedentary males ($p=0.04$). Error bars represent SEM.

Figure 1.


Male
Female
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8000
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4000
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Meters/24 hrs
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Week 1
Week 5
Week 10
Week 20

Figure 2.

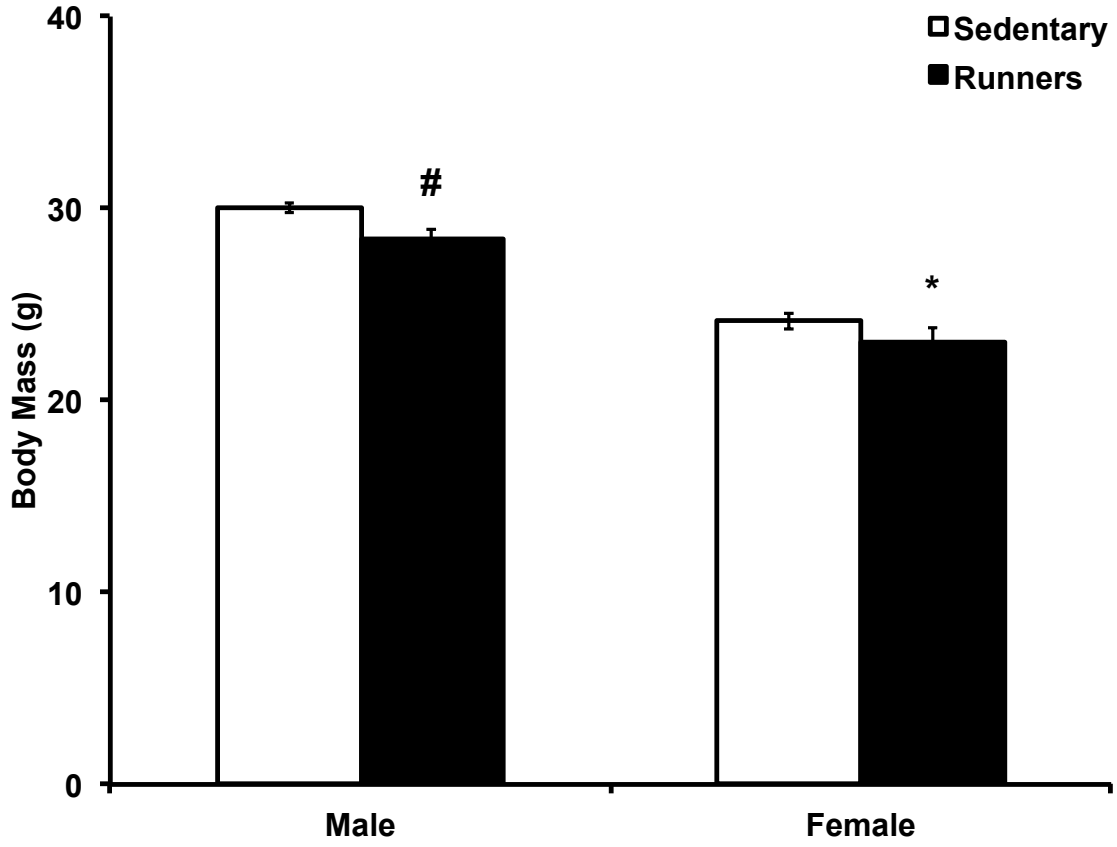
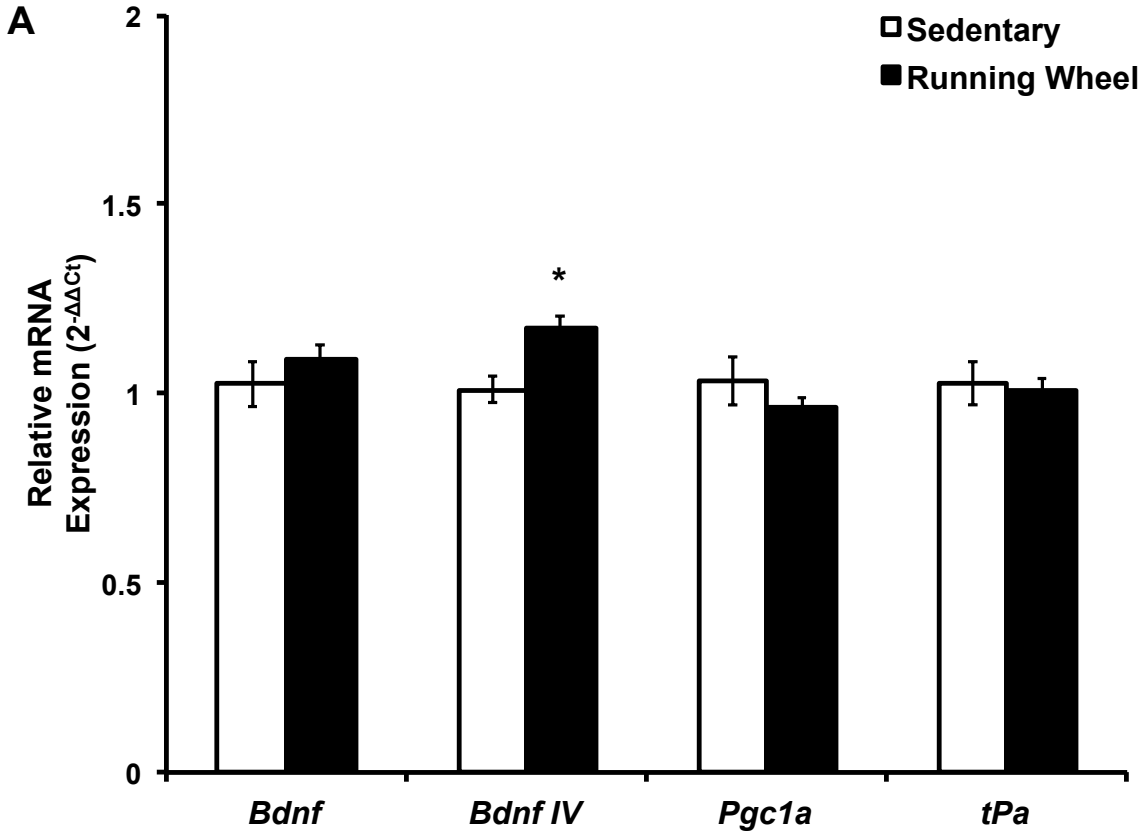
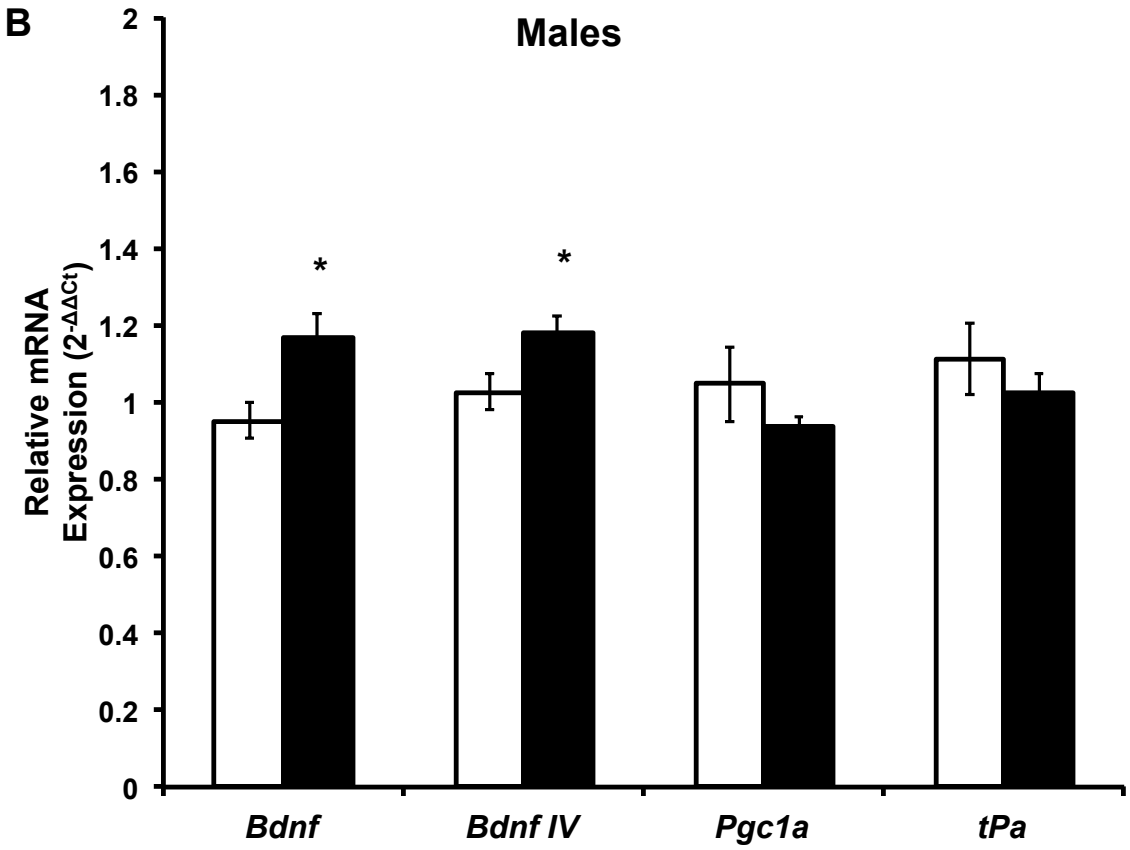


Figure 3.


A
Sedentary
Running Wheel
Relative mRNA Expression ($2^{-\Delta\Delta Ct}$)
2
1.5
1
0.5
0
*
Bdnf
Bdnf IV
Pgc1a
tPa


B
Males
Relative mRNA Expression ($2^{-\Delta\Delta Ct}$)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
Bdnf
Bdnf IV
Pgc1a
tPa

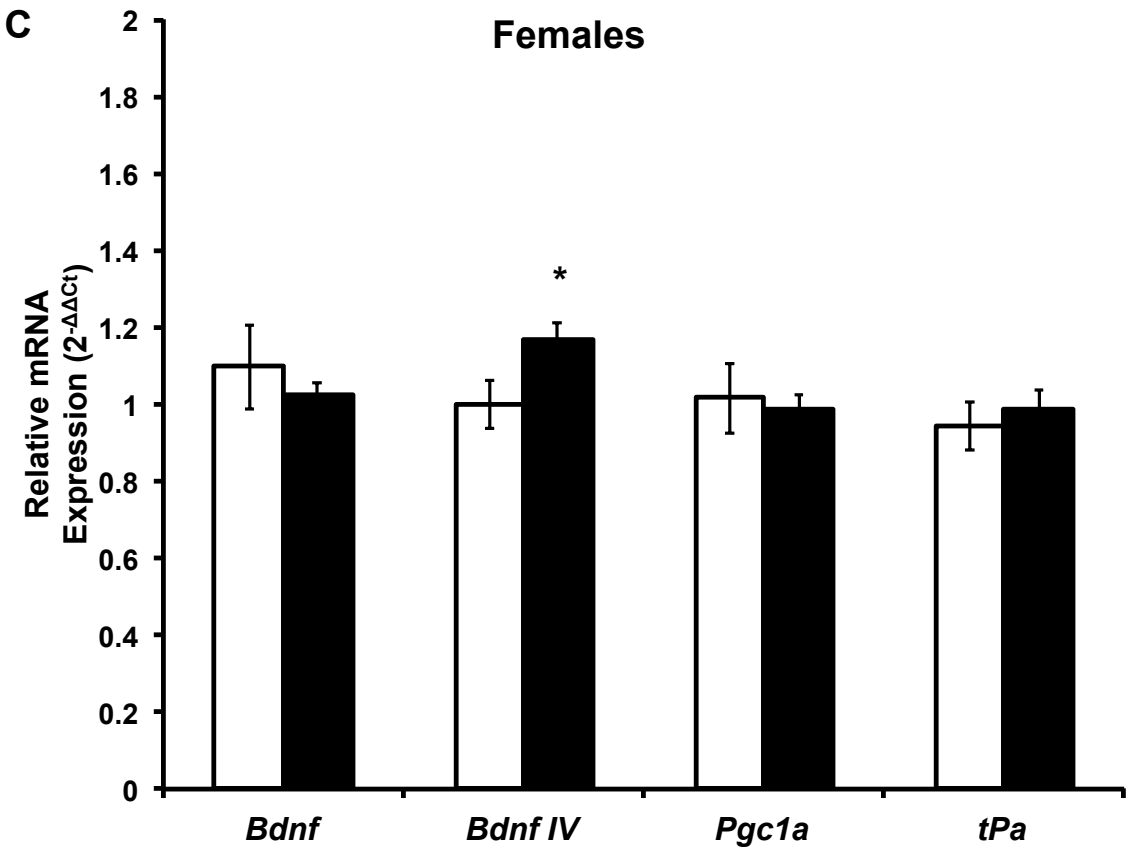
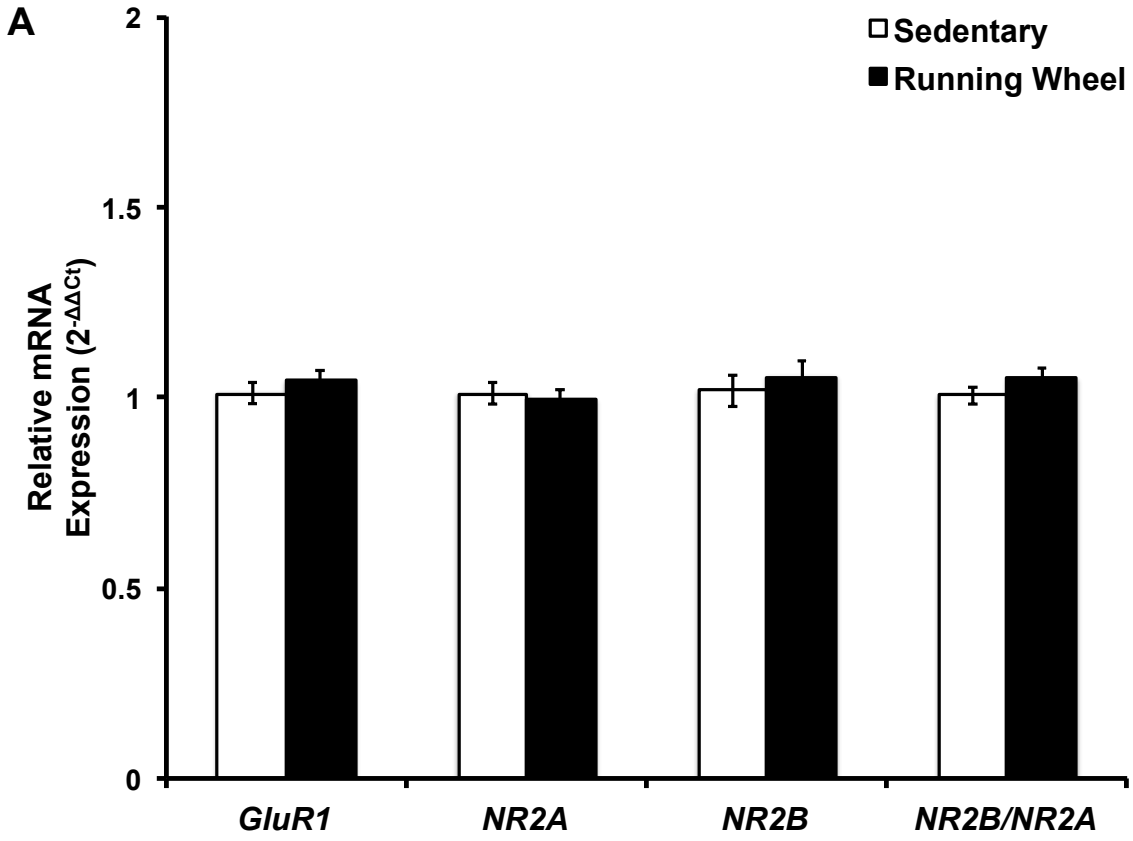
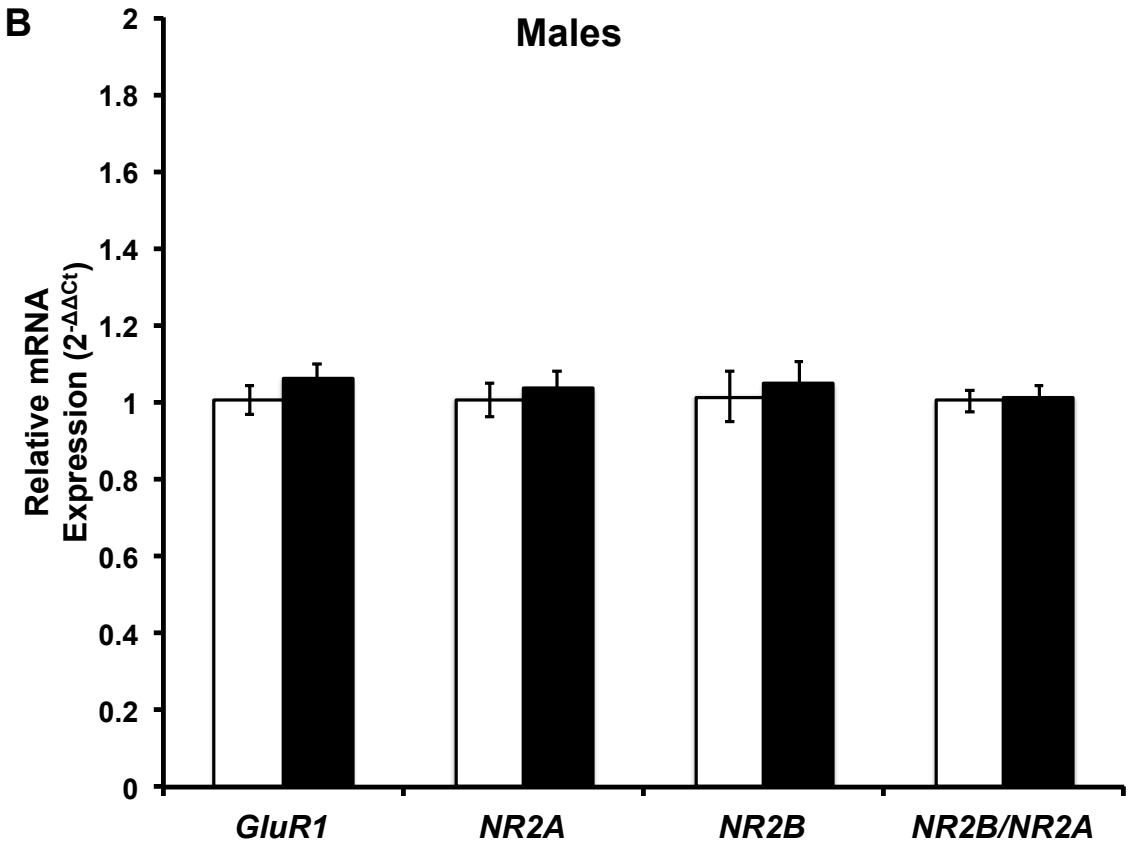

C
Females
Relative mRNA Expression ($2^{-\Delta\Delta Ct}$)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
Bdnf
Bdnf IV
Pgc1a
tPa

Figure 4.


A
Sedentary
Running Wheel
Relative mRNA Expression ($2^{-\Delta\Delta Ct}$)
2
1.5
1
0.5
0
GluR1
NR2A
NR2B
NR2B/NR2A


B
Males
Relative mRNA Expression ($2^{-\Delta\Delta Ct}$)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
GluR1
NR2A
NR2B
NR2B/NR2A

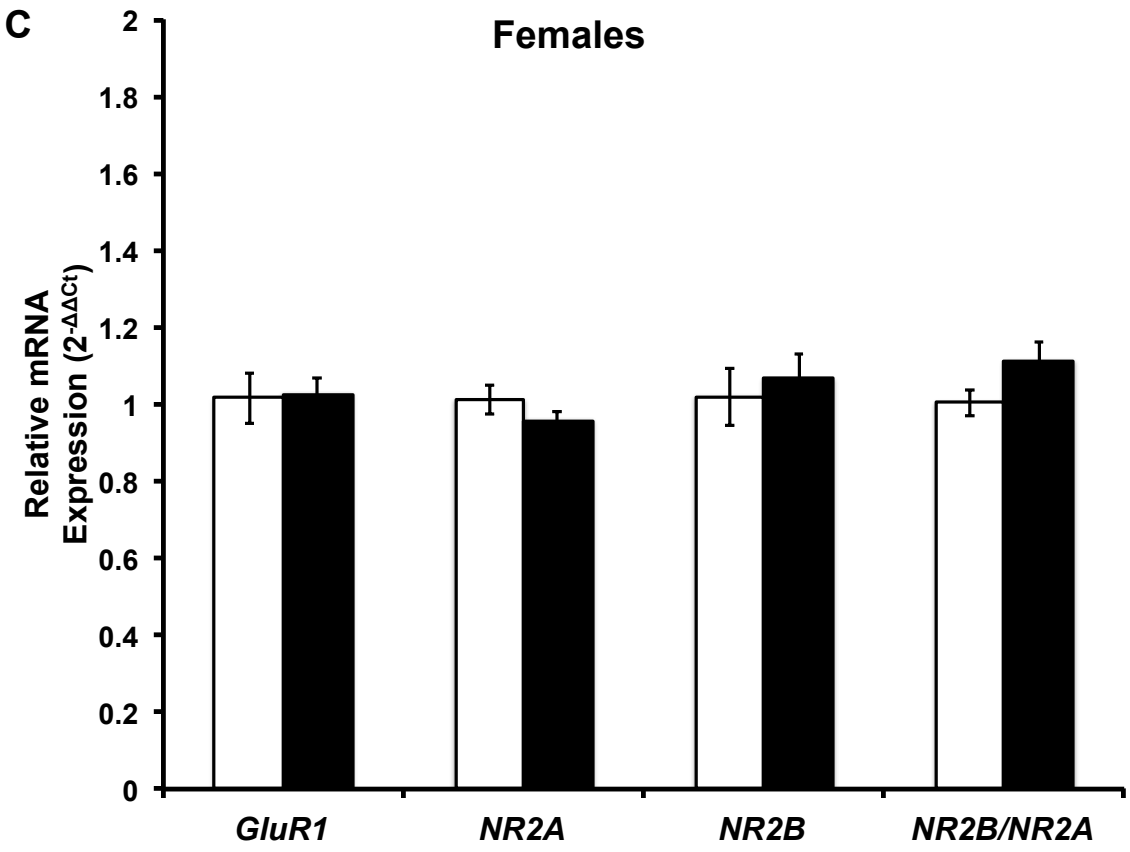
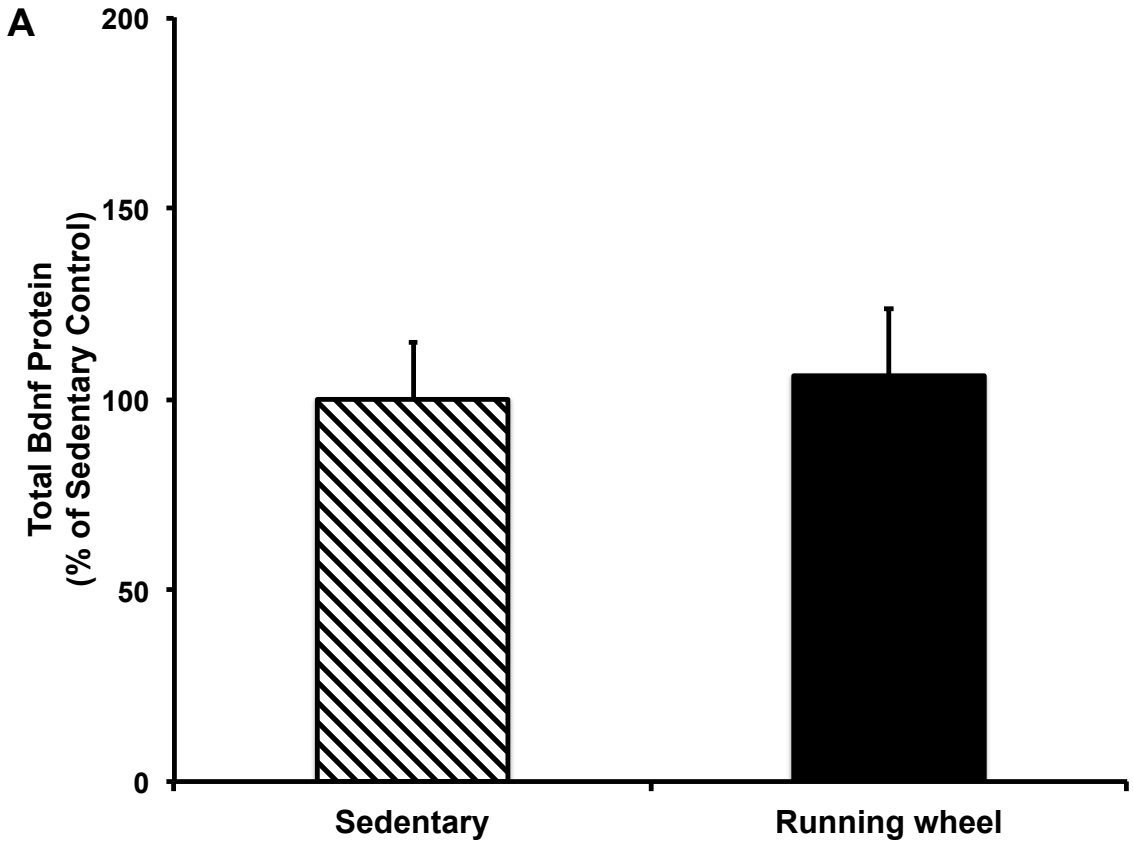

C
Females
Relative mRNA Expression ($2^{-\Delta\Delta Ct}$)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
GluR1
NR2A
NR2B
NR2B/NR2A

Figure 5.


A
200
150
100
50
0
Total Bdnf Protein
(% of Sedentary Control)
Sedentary
Running wheel

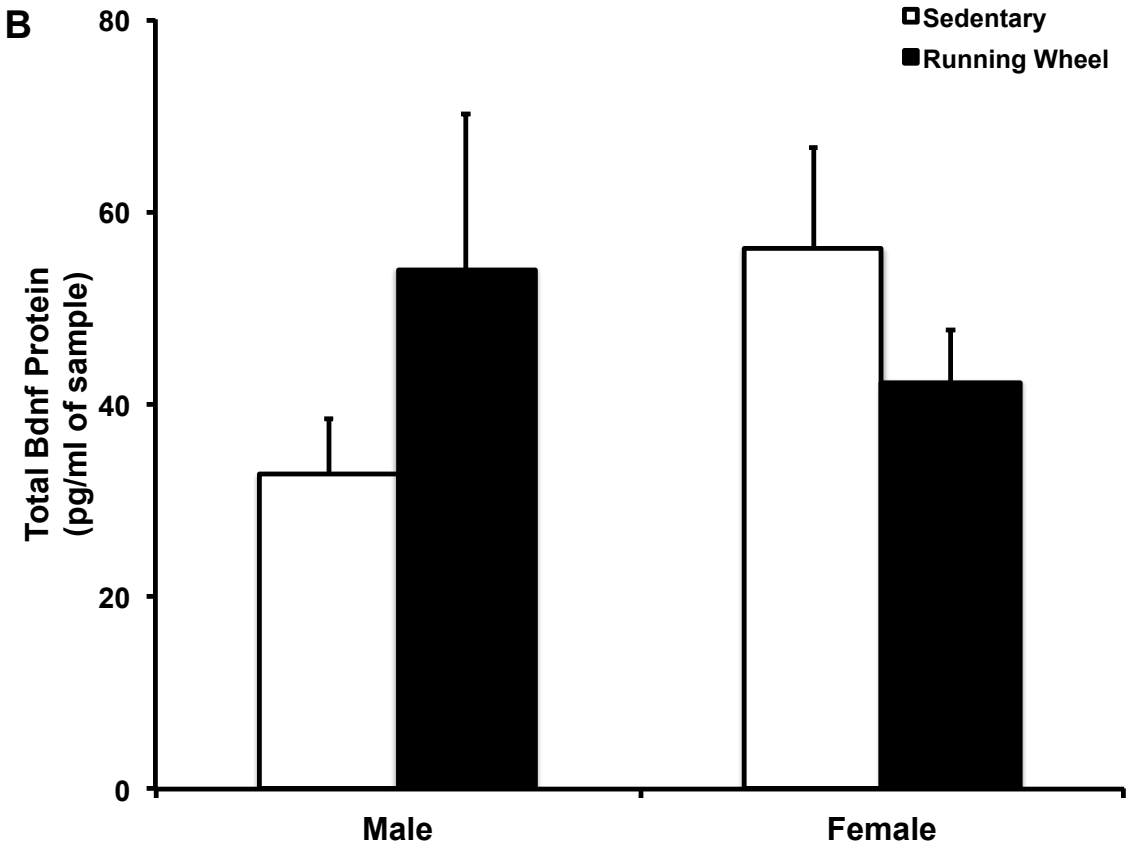
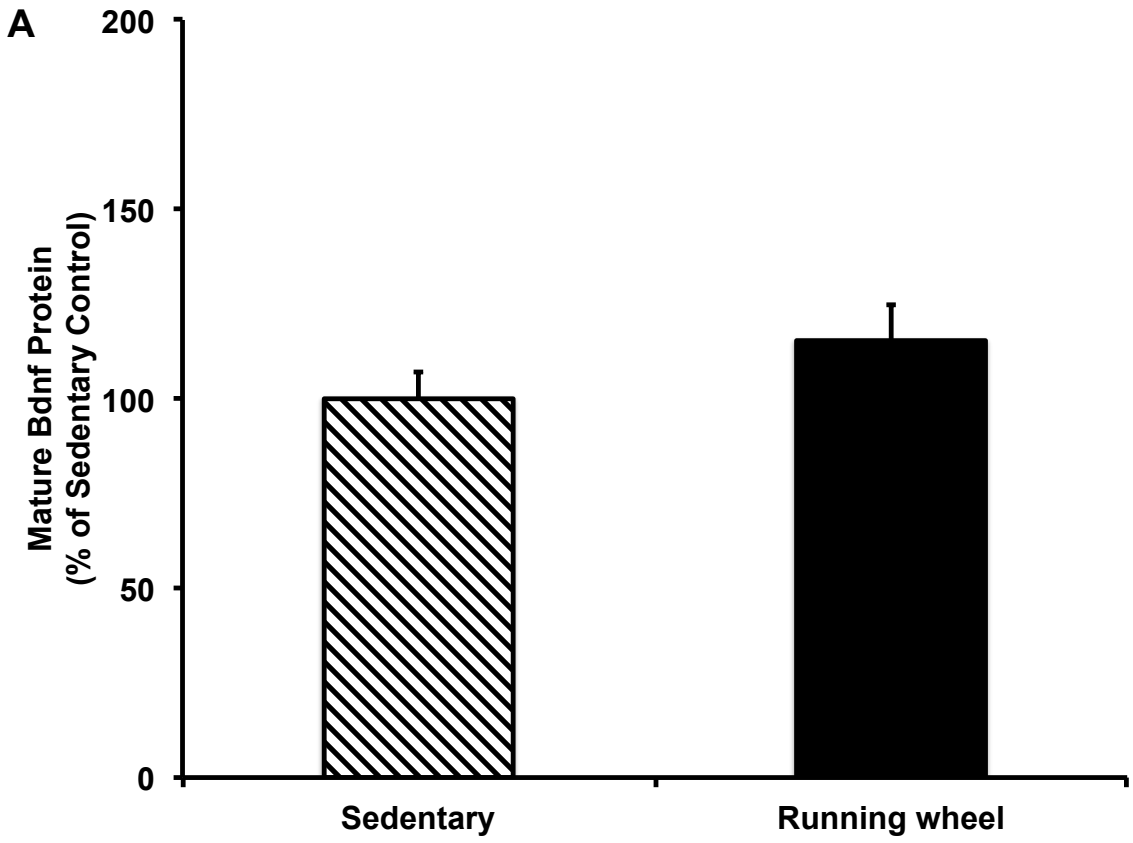
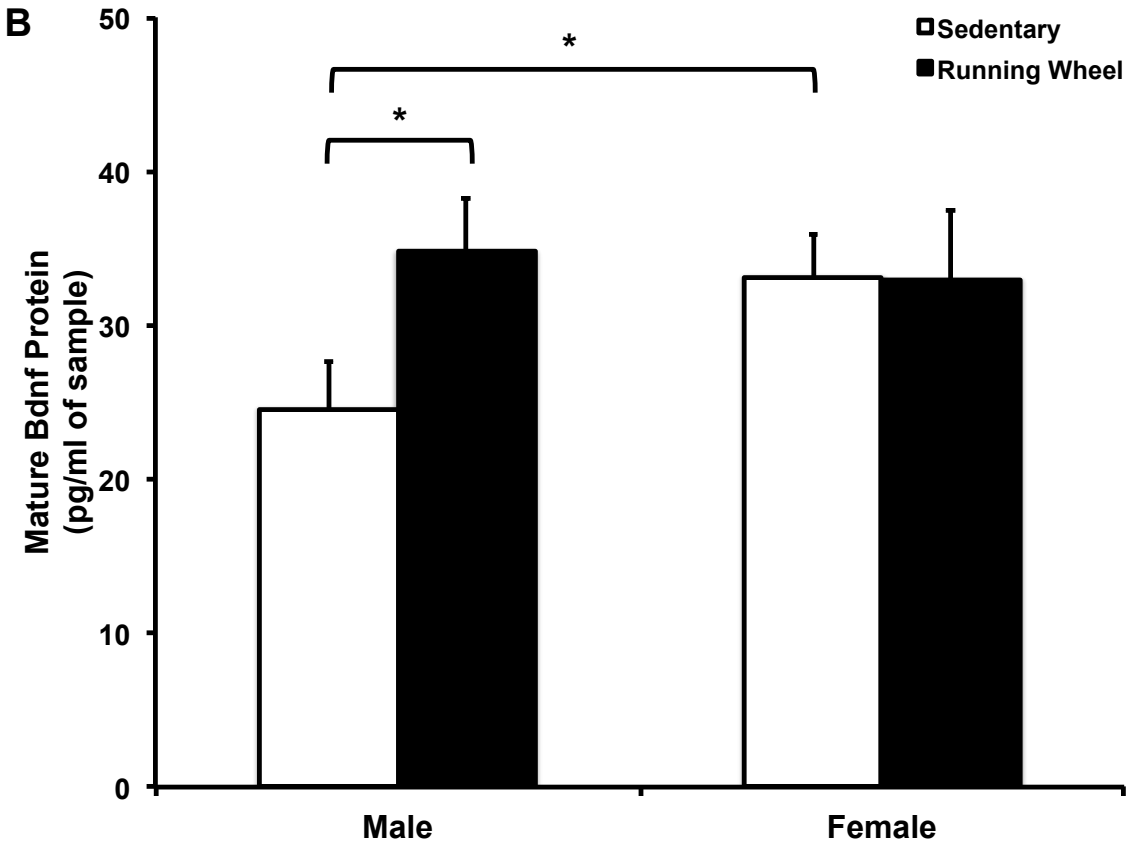

B
80
60
40
20
0
Total Bdnf Protein
(pg/ml of sample)
Sedentary
Running Wheel
Male
Female

Figure 6.


A
200
150
100
50
0
Mature Bdnf Protein
(% of Sedentary Control)
Sedentary
Running wheel


B
50
40
30
20
10
0
Mature Bdnf Protein
(pg/ml of sample)
*
*
Sedentary
Running Wheel
Male
Female

Supplemental Table 1. Primer sequences for genes of interest.

mRNA Target	Primer Sequences
Bdnf total	Primer 1: 5' – CCATAAGGACGCGGACTTGTAC -3' Primer 2: 5' – AGACATGTTTGCGGCATCCAGG -3'
Bdnf IV	Primer 1: 5'- CAGAGCAGCTGCCTTGATGTT -3' Primer 2: 5'- GCCTTGTCCGTGGACGTTTA -3'
Pgc-1a	Primer 1: 5' – GGTGTCTGTAGTGGCTTGATTC -3' Primer 2: 5' – GTTCCCGATCACCATATTCCA -3'
tPa (Plat)	Primer 1: 5' – CAACCAAGACCTCCACGA -3' Primer 2: 5' – CACATCCTTCTGCCCACA -3'
NR2A (Grin2a)	Primer 1: 5' – TGCTCATCACCTCATTCTTCT C – 3' Primer 2: 5' – GATTGACCTCGCTCTGCTC – 3'
NR2B (Grin2b)	Primer 1: 5' – CACAAACATCATCACCCACAC -3' Primer 2: 5' – TTGACTTCTCTGTGCCCTTC – 3'
GLUR1 (Gria1)	Primer 1: 5' – TGGCGAGGATGTAGTGGTA – 3' Primer 2: 5' – AAGAAAAAGGAGAGGCTGGTG – 3'
Gapdh	Primer 1: 5' – AATGGTGAAGGTCGGTGTG – 3' Primer 2: 5' – GTGGAGTCATACTGGAACATGTAG – 3'

Discussion

We found a sex- and transcript-dependent effect of long-term voluntary wheel running on *Bdnf* transcription. Five months of voluntary wheel running increased *Bdnf IV* gene expression but had no effect on total *Bdnf* expression in the combined sample (male & female). Interestingly, when males and females were separated for analysis, we observed an increase in *Bdnf IV* in both males and females and an increase in total *Bdnf* in males only. Moreover, we found that five months of voluntary wheel running increased mature Bdnf protein in males but had no effect in females, which is consistent with total *Bdnf* mRNA and provides strong evidence for sex-dependent effects of long term exercise training on Bdnf expression and processing. These are interesting observations because voluntary wheel running enhances hippocampal plasticity in both males (Farmer *et al.*, 2004) and females (van Praag, Christie, *et al.*, 1999).

Numerous studies have demonstrated that both brief and longer exercise training exposures increase hippocampal Bdnf mRNA and protein expression (Neeper *et al.*, 1996; Molteni *et al.*, 2002; Vaynman *et al.*, 2003; 2004; Berchtold *et al.*, 2005; 2010; Ding *et al.*, 2011; Sartori *et al.*, 2011). In addition, human studies have demonstrated that peripheral BDNF levels are elevated with aerobic exercise training (Szuhany *et al.*, 2015). However, we report here that five months of voluntary wheel running increases total *Bdnf* mRNA and mature Bdnf protein only in male mice. When males and females were combined for analysis, there were no significant effects of exercise on *Bdnf* mRNA or mature protein

expression. The majority of research to date has primarily used only males or only females, making our results difficult to compare to the literature. However, Gallego et al. (2015) reported that 21 days of voluntary running wheel access increased Bdnf protein and mRNA expression in the hippocampus of both male and female adolescent C57Bl/6J mice. Both age and duration of wheel exposure have been reported to influence Bdnf expression (Adlard *et al.*, 2005), which might explain the difference between the results reported in Gallego et al. (2015) and this investigation. A recent meta-analysis concluded that exercise training increases peripheral levels of BDNF in humans, though effect sizes were smaller for studies that included females in the sample (Szuhany *et al.*, 2015). Titterness et al. (2011) reported sex differences in hippocampal LTP following two weeks of voluntary wheel running in adolescent rats, though there were no differences in Bdnf protein expression in either males or females. Other research has demonstrated that voluntary wheel running does increase *Bdnf* mRNA expression in females but the expression is dependent on sex hormones (Berchtold *et al.*, 2001). There is strong evidence that sex hormones are important regulators of Bdnf expression (Carbone & Handa, 2013; Pluchino *et al.*, 2013). In humans, plasma BDNF fluctuates during the menstrual cycle and women who experience normal ovulatory cycles have higher plasma BDNF compared to amenorrhoeic or postmenopausal women (Begliuomini *et al.*, 2007). Further, in male-to-female transsexuals, 12-months of hormone therapy results in reduced serum BDNF (Fuss *et al.*, 2015). These studies suggest a complex relationship between sex hormones and BDNF in humans. Further research with

long-term exercise training in ovariectomized mice is necessary. Potentially, a non-Bdnf pathway plays a more important role in exercise-induced hippocampal plasticity in females compared to males, whereas males may rely more heavily on Bdnf-mediated plasticity.

There is evidence of differential hippocampal Bdnf expression between males and females following acute and chronic stress (Lin *et al.*, 2009). Females have a higher prevalence of mental disorders such as clinical depression and post-traumatic stress disorder, though animal research suggests that chronic stress leads to more structural damage to the male hippocampus (Cahill, 2006). The literature suggesting sex differences in hippocampal adaptations to stress offers another potential explanation for the findings reported here. The animals in this investigation underwent glucose tolerance testing one-day prior to sacrifice. This was done to determine if any whole body metabolic adaptations occurred following five months of voluntary wheel running. The IPGTT was novel to the rodents and required handling and a tail snip and, though we took every precaution to minimize the stress response, the procedure was undoubtedly novel and presented an opportunity for stress. Lin et al. (2009) reported that in response to an acute foot shock, female rats responded with greater Bdnf protein expression in the dentate gyrus whereas stressed and control male rats showed no difference in Bdnf expression. Moreover, rodents that are chronically exercised have a lower stress response to stressful stimuli (Dishman *et al.*, 1997; 1998; Greenwood *et al.*, 2003; 2005) and therefore *Bdnf* expression in sedentary females may have been greater compared to exercise females in response to the

IPGTT stress, masking any observable effect of the chronic physical activity. Though there was no difference between sedentary male and sedentary female total *Bdnf* mRNA expression, there was a significant difference in mature Bdnf protein and a tendency for a difference in total Bdnf protein (p=0.08) between sedentary males and sedentary females.

Interestingly, *Bdnf IV* mRNA was greater in exercised mice compared to sedentary mice, and this effect remained when analyzing sexes separately. *Bdnf IV* mRNA expression is stimulated with neural activity (Martinowich *et al.*, 2003), exercise (Gómez-Pinilla *et al.*, 2010; Intlekofer *et al.*, 2013), and other external stimuli (Lubin, 2011). Remarkably, *Bdnf IV* promoter methylation is reduced with fear learning (Lubin, 2011) and short-term exercise (Gómez-Pinilla *et al.*, 2010), and decreased promoter methylation suggests greater transcriptional activity (Martinowich *et al.*, 2003). This is a potential mechanism mediating the effects of long-term exercise training on *Bdnf IV* transcription. The finding that both males and females had increased expression of *Bdnf IV*, though only males had higher total *Bdnf* suggests that exercise stimulates sex-specific up- and/or down-regulation of transcript-specific *Bdnf* gene expression.

The elevation in Bdnf protein in males was limited to mature Bdnf with no difference in total Bdnf, suggesting that five months of voluntary wheel exposure selectively increases expression of the mature plasticity-promoting Bdnf isoform. Sartori et al. (2011) reported that 28 days of voluntary wheel running selectively increased mature Bdnf with no difference in the immature proBdnf in male

C57/Bl6 mice. In contrast, Ding et al. (2011) reported that seven days of wheel exposure increased both mature and proBdnf in the rat hippocampus. Differences in animal model and exercise duration likely explain the differences between our findings and those of Ding and colleagues (2011).

Curiously, five months of voluntary wheel running did not impact the other mRNA targets measured in the present study. *tPa* and *Pgc-1a* mRNA are reportedly increased with voluntary wheel running (Sartori *et al.*, 2011; Steiner *et al.*, 2011), an effect we did not observe. tPa has been shown to influence the beneficial effects of exercise on hippocampal function and is known to be an important enzyme in the cleavage of apoptotic proBdnf to generate the mature and plasticity-promoting mature Bdnf (Pawlak *et al.*, 2005; Sartori *et al.*, 2011). Interestingly, Sartori et al. (2011) also used C57Bl/6J mice and qPCR to demonstrate that voluntary wheel running increases *tPa* expression in the hippocampus. Animals in the Sartori et al. (2011) investigation were only provided access to a voluntary running wheel for 28 days. Longer exposure to a voluntary running wheel may result in a return to control levels of *tPa*. Pgc-1a is a co-transcription factor that regulates mitochondrial biogenesis and when co-expressed with other tissue- and temporal-specific transcription factors, Pgc-1α stimulates the transcription of genes necessary for mitochondrial biogenesis (Finck, 2006). Mitochondrial biogenesis in the rodent hippocampus has been observed following exercise training (Steiner *et al.*, 2011) and we recently reported that *in utero* exercise exposure increases *Pgc-1α* expression in offspring hippocampus (Venezia *et al.*, 2015) (Appendix B). In the current investigation,

we observed no effect of long-term wheel running on *Pgc-1α.* Steiner et al. (2011) reported an increase in hippocampal *Pgc-1α* following an eight-week treadmill exercise protocol. Importantly, forced and voluntary exercise are distinct forms of exercise, generally associated with different levels of stress hormones (Yanagita *et al.*, 2007; Hayes *et al.*, 2008; Liu *et al.*, 2009; Ke *et al.*, 2011), and volume and intensity of exercise (Hayes *et al.*, 2008; Leasure & Jones, 2008). Indeed, voluntary and forced exercise induce both similar and distinct structural and functional adaptations to the rodent brain (Burghardt *et al.*, 2004; Ploughman *et al.*, 2005; Hayes *et al.*, 2008; Leasure & Jones, 2008; Liu *et al.*, 2009; Toscano-Silva *et al.*, 2010; Ke *et al.*, 2011; Kinni *et al.*, 2011), which might explain the discrepancy between our data and Steiner et al (2011). We also observed no influence of chronic wheel running on glutamate receptor subunit expression. Short-term exposure to a voluntary running wheel increases mRNA expression of the *NR2B* subunit of the NMDA glutamate receptor and higher expression of this subunit is associated with a more plastic synapse (Molteni *et al.*, 2002; Farmer *et al.*, 2004). There is limited and inconsistent (increases, decreases, no effect) data on the influence of exercise on *GluR1* and *NR2A* subunit mRNA expression (Molteni *et al.*, 2002; Dietrich *et al.*, 2005; Ni *et al.*, 2009; Real *et al.*, 2010). Potentially, due to the long duration of running and the steady decline in wheel activity over the course of the five months, the stimulus was not intense enough to maintain elevated mRNA expression of plasticity-associated genes. Further, differences in mRNA expression of plasticity-associated genes may have been observed if we investigated specific

hippocampal subfields (dentate gyrus, CA1, and CA3) instead of whole hippocampal homogenates. Hippocampal subfields contain specific cell types and varying levels of sensitivity and adaptations to stimuli including exercise (Andersen *et al.*, 2006; Voss *et al.*, 2013). Future investigations should utilize additional methods of mRNA detection such as in situ hybridization.

A limitation of our investigation was that the control group was not housed with a locked running wheel. The differences observed in Bdnf mRNA and protein expression were potentially due to enriched housing or the combined effects of enriched housing and running. Sartori et al. (2011) observed greater mature Bdnf protein in mice housed with a locked running wheel compared to mice housed in standard cages without a wheel, demonstrating that the presence of a wheel can influence Bdnf expression independent of running. Importantly, our data still demonstrate that long-term housing with a freely rotating voluntary running wheel influences Bdnf expression and processing differently between sexes.

Summary. The present data suggest that long-term voluntary exercise has limited and sex-dependent effects on hippocampal mRNA and Bdnf protein expression. Due to the limited effects of long-term voluntary wheel running observed in our investigation, we speculate that voluntary wheel running volume and/or intensity might need to be maintained or even manipulated (i.e., increase intensity) to most effectively maintain hippocampal plasticity-associated gene and protein expression. This might explain why we saw a greater benefit of exercise

in males, which maintained their running volume better than females. Voluntary wheel running is a good model for unstructured leisure-time physical activity but might not be the best model for exercise training, which is generally associated with structured frequency, intensity, and duration components. Forced exercise, though associated with elevated stress hormones (Ploughman *et al.*, 2005; Yanagita *et al.*, 2007; Hayes *et al.*, 2008; Liu *et al.*, 2009; Ke *et al.*, 2011), might be an alternative to enhance brain health and plasticity (Hayes *et al.*, 2008; Leasure & Jones, 2008; Liu *et al.*, 2009; Toscano-Silva *et al.*, 2010; Kinni *et al.*, 2011; Lin *et al.*, 2012) in the long-term if declining voluntary running volume and intensity is limiting plasticity, which is yet to be determined. Future research should examine the effectiveness of long-term forced exercise due to the high levels of stress associated with this model of exercise.

Competing interest

None declared

Funding

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Authors' contributions.

ACV, LMG, EES, and SMR designed the study; ACV, LMG, and RMS collected the data; data analysis, preparation of figures, and drafting the manuscript was done by ACV; ACV, LMG, RMS, EES, and SMR edited and revised this manuscript; and all authors approved the final version.

Chapter 4.

Aim #2. Determine the effect of acute exercise and exercise intensity on GluR1 phosphorylation, the expression of specific plasticity-associated genes, and novel object location memory in three-month old C57BL/6J mice.

Aim #3: Determine if acute high-intensity exercise increases anxiety-like behavior in the open field task and if this behavioral phenotype is attenuated with pre-treatment with the selective noradrenergic neurotoxin DSP-4.

Title: Acute Forced Exercise Increases Expression of *Bdnf IV* and Induces Anxiety-Like Behavior in C57BL/6J Mice.

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Neuroscience and Cognitive Science Program, University of Maryland, College Park, MD[2]

Department of Psychology, University of Maryland, College Park, MD[3]

Department of Biology, University of Maryland, College Park, MD[4]

Introduction

Exercise is an effective way to maintain and improve brain health, and research in humans and rodents indicates that the hippocampus, a brain region important for memory formation and the response to emotional and stressful situations (Moser & Moser, 1998; Strange *et al.*, 2014), is particularly sensitive to physical activity and exercise (Voss *et al.*, 2013). Adaptations observed in the hippocampus with exercise training include neurogenesis (van Praag, Christie, *et al.*, 1999; van Praag, Kempermann, *et al.*, 1999), dendritic arborization (Stranahan *et al.*, 2007; Lin *et al.*, 2012), and increased amplitude and reduced threshold of long term potentiation (LTP; van Praag, Christie, *et al.*, 1999; Farmer *et al.*, 2004), a long-lasting enhancement of synaptic strength and a leading candidate for the molecular mechanism of memory formation (Malenka, 1999). Though numerous studies have investigated the mechanisms that mediate these beneficial adaptations in response to exercise training, the mechanisms that directly mediate enhanced synaptic efficacy following exercise have not been identified. Moreover, the molecular events that occur in response to a single bout of acute exercise have not been thoroughly investigated. Chronic exercise, which has been the focus of the majority of research to date, is the accumulation of individual acute bouts of exercise, each of which can be optimized to enhance the benefits of chronic exercise training on brain health.

The enhancement of synaptic transmission mediating LTP is a result of an increase in the number of synaptic AMPA receptors (AMPARs) that mediate the

majority of the response to synaptically-released glutamate (Huganir & Nicoll, 2013). AMPARs are heterotetramers composed of GluR1, GluR2, GluR3, and GluR4 subunits, with the adult brain primarily containing AMPARs composed of GluR1/GluR2 and GluR2/GluR3 subunit combinations. The threshold for LTP can be reduced by phosphorylation of the C-terminal tail of the GluR1 subunit and subsequent insertion of GluR1 containing AMPARs into the perisynaptic membrane, making this receptor more available for subsequent migration and "capture" by the synapse (Kessels & Malinow, 2009). Phosphorylation of serine 831 (Ser831) on GluR1 by CAMKII increases the frequency of synaptic "capture" of AMPARs and ion channel open probability (Kessels & Malinow, 2009; Huganir & Nicoll, 2013), while phosphorylation of serine 845 (Ser845) by PKA increases perisynaptic insertion and decreases AMPAR internalization (Oh *et al.*, 2006; Santos *et al.*, 2009). In fact, mutations that prevent phosphorylation of Ser831 and Ser845 compromise synaptic plasticity (Lee *et al.*, 2003) and prevent the lowered threshold for LTP and learning triggered by exogenous catecholamines (Hu *et al.*, 2007). Moreover, mimicking phosphorylation with knock-in mutations reduces the threshold for LTP and occludes the effects of exogenous norepinephrine (Makino *et al.*, 2011). Understanding the mechanisms and stimuli that cause phosphorylation and membrane insertion of GluR1 is a focus of intense investigation and exercise has the potential to be a practical, non-invasive strategy to achieve this molecular event. In fact, following cortical infarction, exercise training can induce Ser845 phosphorylation in the rodent cortex (Mizutani *et al.*, 2015), indicating that physical activity has the potential to

induce this adaptation. Whether or not acute exercise can achieve this in the healthy hippocampus is unknown. The reduced threshold for LTP (Farmer *et al.*, 2004) and learning (Intlekofer *et al.*, 2013) observed following exercise training in healthy rodents may be mediated by phosphorylation of important sites on the C-terminal tail of GluR1; however, this has not been thoroughly investigated in the rodent hippocampus. Acute exposure to psychological stress or peripheral injections of epinephrine increase phosphorylation of Ser845 on GluR1 and reduce the threshold for LTP and learning (Hu *et al.*, 2007). Importantly, acute bouts of exercise also increase release of peripheral epinephrine and central norepinephrine (Pagliari & Peyrin, 1995a; Zouhal *et al.*, 2008). Like acute exposure to psychological stress, a single acute bout of exercise has the potential to influence the phosphorylation status of this important AMPA receptor subunit and might be the mechanism by which acute bouts of exercise strengthen memory formation (Lambourne & Tomporowski, 2010; Intlekofer *et al.*, 2013; Roig *et al.*, 2013).

Brain-derived neurotrophic factor (Bdnf) is a critical protein for functional and structural hippocampal plasticity and is upregulated with chronic and short-term exercise training (Vivar *et al.*, 2012; Voss *et al.*, 2013). Though the importance of Bdnf is well known (Park & Poo, 2013), the extent to which individual bouts of exercise influence Bdnf expression is not understood. Research investigating “acute” exercise in rodents generally follows a period of treadmill running familiarization or is a voluntary running wheel exposure lasting from several hours to multiple days. Bdnf expression is important for different

stages of memory formation (Bekinschtein *et al.*, 2014) so understanding the temporal dynamics of Bdnf expression and the stimuli that upregulate its expression is important to most effectively utilize exercise or exercise-like stimuli to enhance memory. In addition, transcription of other plasticity-associate genes, such as glutamate receptor subunits, may also be influenced by acute bouts of exercise and identifying these genes could shed light on the mechanisms that lead to memory enhancement following acute and chronic exercise.

Though research in humans suggests acute exercise can improve memory (Lambourne & Tomporowski, 2010; Roig *et al.*, 2013), this has not been adequately investigated in rodents. Since rodents are commonly used as models to understand the mechanisms that drive behavioral adaptations, it is important to understand the behavioral modifications occurring in response to acute exercise if this model is to be used appropriately. We investigated the influence of a single bout of acute treadmill exercise on GluR1 phosphorylation, plasticity-associated gene expression, and performance on a one-trial spatial memory task and a locomotor-dependent anxiety test. Further, because exercise stimulates the release of central and peripheral catecholamines (Pagliari & Peyrin, 1995a; Chatterton *et al.*, 1996; Zouhal *et al.*, 2008), which are known to influence anxiety like behavior (Goddard *et al.*, 2010), we also wanted to understand how noradrenergic signaling influences animal behavior. To explore this relationship, we utilized a pharmacological approach to compromise the central noradrenergic system prior to acute exercise and assessment of anxiety-like behavior.

Methods

Mouse model. Three-month old male C57BL/6J mice (Jackson Laboratories (Bar Harbor, ME, USA) were used in this investigation. This mouse strain is commonly used to study the impact of exercise on brain phenotypes and in our lab displays avid treadmill running activity and normal physiological adaptations to exercise (e.g. improved glucose metabolism, lower body mass, enhanced markers of oxidative capacity, etc.; Ludlow *et al.*, 2012; Guth *et al.*, 2013). All mice were group housed and cared for by UMD veterinary staff. Mice were kept on a 12hr light/12hr dark cycle and provided standard rodent chow. All protocols were approved by the University Institutional Animal Care and Use Committee.

Overview and Treadmill Protocol: To address the question of how acute exercise influences markers of hippocampal plasticity, mice were randomly separated into three groups: 1) treadmill without exercise (CON; n=12); 2) moderate-intensity acute treadmill exercise (MOD; n=12); 3) high-intensity acute treadmill exercise (HI; n=12). For three days leading up to the experiment, mice were placed on the stationary treadmill for five minutes per day, during which the electrical stimulus grid at the end of the treadmill belt was activated, so that all mice were familiarized with the stimulus and treadmill-testing environment. During active treadmill running, the stimulus grid provides a weak foot shock, which causes an involuntary muscle contraction that encourages running**.** Tactile stimulation to the tail was used to encourage mice to run prior to touching the stimulus grid, which reduced the number of stimulus grid touches (unpublished observation)**.** On day

four, MOD and HI group mice were placed on the treadmill, one at a time, and the acute bout of exercise was initiated. Each mouse underwent a six-minute warm up, where the first minute was a no-exercise treadmill exposure; thereafter the treadmill belt began to move at 5 m/min, increasing 1 m/min every minute for five minutes. The treadmill speed was then incrementally increased to the group-appropriate speed and the mouse ran for 30 minutes at this pace. In our laboratory, this warm up is an effective method to encourage mice to run on the treadmill without multiple acclimation trials. The MOD group ran for 30 minutes at 12 m/min at 0% grade; this stimulus has been reported to be ~75% of VO_{2max} in adult C57BL/6J mice (Schefer & Talan, 1996). The HI group ran for 30 minutes at a speed ranging from 15-17 m/min at 0% grade, depending on running ability; this speed has been reported to be ~80% of VO_{2max} in adult C57BL/6J mice (Schefer & Talan, 1996). Mice in the CON group were placed on the stationary treadmill for 36 minutes with the electrical stimulus grid activated. Immediately following the treadmill bout, mice were sacrificed for gene expression and biochemical analysis.

Tissue Processing: Mice used for mRNA and protein analysis were sacrificed by decapitation under isoflurane anesthesia immediately following the acute exercise bout. The hippocampus was halved, isolated, and immediately frozen in liquid nitrogen. For western blot analysis, the hippocampus was sonicated in 1% SDS, boiled for 10 minutes (Hu *et al.*, 2007), and stored at -80°C. Protein concentration was determined by spectrophotometry using the BCA Protein Assay (Pierce®, Rockford, IL). RNA was isolated from the remaining half

hippocampus. Samples were homogenized in a glass Dounce tissue homogenizer followed by RNA isolation using TRI Reagent (Life Technologies, Grand Island, NY, USA). RNA quantity and purity were assessed by UV spectroscopy.

Western Blotting: Twenty-five µg of protein was loaded onto polyacrylamide gels and electrophoresed, followed by transfer to nitrocellulose membranes and immunoblotting. Nitrocellulose membranes were incubated with anti-phospho-GluR1 (Ser845; Millipore or Ser831; Millipore, Billerica, MA) antibodies, stripped in a glycine- HCl stripping solution, and re-probed with an anti-GluR1 antibody (Millipore). Although the short time between the initiation of the exercise bout and sacrifice (30 minutes) should prevent any changes in total GluR1 translation, we also blotted for total GluR1 protein followed by stripping and re-probing for the neuronal nuclear marker NeuN (Millipore). Appropriate fluorescent secondary antibodies were used for detection. A Typhoon scanner (GE Healthcare UK Limited, Buckinghamshire, England) was used to digitize the fluorescent signal. Levels of phosphorylation, expressed as the ratio of phospho-GluR1 divided by total GluR1 intensity from the same lane, were used for statistical analysis.

To confirm that peripheral epinephrine increases hippocampal GluR1 Ser845 phosphorylation, a subsample of mice were injected with saline (10ml/kg; n=3) or epinephrine (0.5mg/kg at 10 ml/kg; n=4) and sacrificed 15 minutes post-injection. Hippocampi were sonicated in lysis buffer [50 mM Tris-HCl (pH 7.4),

150 mM NaCl, 1% NP-40, protease inhibitor cocktail] and immunoblotted as described above.

Gene Expression: One µg of total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time quantitative PCR (qPCR) was used to assess mRNA expression of total brain derived neurotrophic factor (*Bdnf*; exon IX), *Bdnf* exon IV (*Bdnf IV*), *GluR1*, *NR2A*, *NR2B*, *Gapdh*, and *ActB* (*Gapdh & ActB*; expression controls; primer sequences listed in Appendix A). Primer:probe assays were purchased pre-made [*GluR1 (Gria1), NR2A (Grin2a), NR2B (Grin2b), Gapdh, ActB*] or designed (*Bdnf IX, Bdnf IV*) for the mRNA sequence of each gene using Integrated DNA Technologies' PrimeTime qPCR Assay designer. All primer pairs except *Bdnf* total spanned exons to prevent amplification of genomic DNA. Because *Bdnf* total is represented by amplification of only exon IX, this primer pair could not span exons. Efficiency for each primer:probe assay was determined prior to use. qPCR data were normalized to the geometric mean of *Gapdh* and *ActB* using the -ΔΔCt method (Vandesompele *et al.*, 2002; Schmittgen & Livak, 2008) and expressed as fold induction ($2^{-\Delta\Delta Ct}$) of mRNA expression compared to the control group (1.0-fold induction).

Object Location Memory: A subset of three-month old male C57BL/6J mice were tested on the object location memory task immediately following the acute bout of exercise or no-exercise treadmill exposure. The treadmill familiarization approach was the same as described above. Mice in the treadmill control group

(n=15) sat on the stationary treadmill for 36 minutes while mice in the exercise group (n=15) ran on the treadmill at 15 to 17 m/min for 30 minutes following a six-minute warm-up. The procedures for the object location task were adapted from Barker and Warburton (Barker & Warburton, 2011). Prior to test day, mice were exposed to the testing apparatus (43x43x21.5 cm open field box) for five minutes/day over two consecutive days to acclimate them to the behavioral procedure. On the test day, immediately following the treadmill exposure, mice were placed in the testing apparatus for the familiarization phase of the task and allowed to explore the box and two identical objects (small, Duplo blocks) for five minutes and then returned to their home cages for a fifteen-minute inter-trial interval. Following this interval, mice were returned to the testing apparatus for the test phase when they were presented with one of the objects from the initial exposure phase and a third object that was identical to the initial exposure phase objects, though it was moved to a different location of the box. Mice explored the box and the two objects for five minutes. The left/right position of the moved object was counterbalanced between mice. Behavior during the familiarization and test phases was monitored using EthoVision XT 11 Behavioral Tracking Software (Noldus, Leesburg, VA), which provides automatic tracking, analysis, and storage of animal activity and behavior. Object interaction (time spent with sample objects and number of interactions), latency to approach objects, and total distance moved were recorded for each mouse.

Open Field Behavior Task: An additional subset of three-month old male C57BL/6J mice were used to assess anxiety-like behavior and the influence of

noradrenergic signaling on behavior following acute exercise. Mice were separated into four groups: 1) Stationary Treadmill – Saline (CON-SAL; n=9); 2) Stationary Treadmill – DSP-4 (CON-DSP4; n=10); Treadmill Exercise – Saline (EX-SAL; n=8); Treadmill Exercise – DSP-4 (EX-DSP4; n=9). Mice underwent the same treadmill familiarization and running as described above and ran at a treadmill speed between 15 and 17 m/min depending on running ability. Immediately after the acute bout of exercise or no-exercise treadmill exposure, performance on the open field task was assessed. Immediately following the treadmill exposure, mice were placed in the testing apparatus (43x43x21.5 cm field box) and allowed to explore for 15 minutes. Behavior was monitored using the EthoVision XT 11 Behavioral Tracking Software. Total distance moved, time spent in central and peripheral zones, and time spent grooming were recorded and separated into five minute blocks (0-5 minutes, 5-10 minutes, 10-15 minutes).

N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4): DSP-4 is a selective neurotoxin that specifically lesions the locus coeruleus (LC) noradrenergic (NA) system. DSP-4 dramatically reduces tissue levels of norepinephrine in regions innervated by the LC, such as the hippocampus (Ross, 1976; Ögren *et al.*, 1980; Jonsson *et al.*, 1981; Archer *et al.*, 1982; Anisman *et al.*, 1984; Zahniser *et al.*, 1986; Bennett *et al.*, 1990; Scullion *et al.*, 2009; Szot *et al.*, 2010), though leaves non-LC NA neurons and serotonergic and dopaminergic systems essentially unaffected (Ross & Stenfors, 2014). This chemical is commonly used to irreversibly disrupt central NA signaling because it easily crosses the blood brain barrier and therefore can be injected systemically (Ross & Stenfors, 2014).

Importantly, DSP-4 treatment does not alter running activity in rodents (Garcia *et al.*, 2003). DSP-4 (Sigma Aldrich) was prepared in 0.9% saline and a single 50 mg/kg dose was delivered by IP injection in a volume of 10 ml/kg; this dose is frequently used in both rats and mice and is effective in depleting hippocampal norepinephrine (Ross & Stenfors, 2014). Control mice received a single IP injection of 0.9% saline. Solutions were prepared for five animals and any remaining solution was discarded. Injections were delivered within 15 minutes of solution preparation and was kept out of the light. Animals received injections seven days prior to treadmill familiarization (10 days prior to experimental treadmill day). This dose of DSP-4 and interval between injection and task performance results in >90% reduction in hippocampal norepinephrine in C57BL/6J mice (Scullion *et al.*, 2009).

Statistical Analysis: To determine differences in GluR1 protein expression/ phosphorylation and mRNA expression between groups we used a one-way analysis of variance with Tukey's post hoc comparisons when appropriate ($p<0.05$ considered statistically significant). Object location memory performance was analyzed with a repeated measures ANOVA and Sidak's multiple comparison test when appropriate. Open field behavior data was analyzed using a two-way ANOVA (treadmill exposure x drug treatment) and Tukey's post hoc comparisons when appropriate ($p<0.05$ considered statistically significant).

Results

GluR1 phosphorylation: We were able to confirm that an IP injection of epinephrine was sufficient to induce phosphorylation of Ser845 ($t_{(5)}$= 3.048; p=0.03) but did not influence Ser831 phosphorylation or GluR1 protein expression (Fig. 7). In contrast, an acute bout of high- or moderate-intensity treadmill running did not influence phosphorylation of Ser845 or Ser831 (Fig. 8).

Glutamate Receptor mRNA: There was no effect of moderate- or high-intensity acute exercise on *GluR1*, *NR2A*, or *NR2B* mRNA expression (Fig. 9).

Bdnf mRNA: There was an intensity-dependent significant effect of acute treadmill exercise on *Bdnf IV* expression ($F_{(2, 32)}$=3.79; p=0.03; Fig. 10A). High intensity treadmill exercise resulted in higher mRNA expression compared to controls (adjusted p=0.03). There was no significant effect of acute exercise on total *Bdnf* mRNA expression (Fig. 10B).

Object Location Task: There were no main effects or interaction effect of acute exercise or phase of the task on total time spent exploring the two objects (Fig. 11A). During the test phase, there was no significant difference between controls and exercisers in time spent with the newly moved object relative to time spent with both objects (Fig. 11B). There was a main effect of acute exercise on total distance moved ($F_{(1,28)}$=14.06; p=0.008) but no main effect of test phase. There was a tendency for an interaction effect between acute exercise and test phase on total distance moved ($F_{(1,28)}$=3.684; p=0.07). Mice exposed to high-intensity treadmill running moved significantly less (total distance traveled) during the

familiarization phase compared to treadmill controls (Fig. 12A; p=0.0003). There was a main effect of acute exercise on number of interactions with the objects ($F_{(1,28)}$=4.553; p=0.04) and an interaction effect ($F_{(1,28)}$=6.938; p=0.01) but no main effect of test phase. The treadmill exercisers interacted with the two objects significantly less frequently than treadmill controls during the familiarization phase (Fig. 12B; p=0.003).

Open Field Task:

0-5 minutes: During the first five minutes of the open field task we observed a main effect of exercise ($F_{(1,32)}$ = 33.09; p <0.0001) and an interaction between drug and exercise ($F_{(1,32)}$ = 4.25; p=0.048) but no main effect of drug (Fig. 13A). Post hoc analysis revealed that EX-SAL mice had significantly lower total distance moved during the first five minutes compared to CON-SAL mice (adjusted p <0.0001) (Fig 13A). There was no significant difference between CON-DSP4 and EX-DSP4, though EX-DSP4 had significantly less total distance traveled than CON-SAL (adjusted p=0.003) (Fig. 13A). We also observed a main effect of exercise on time spent grooming during the first five minutes in the open field task ($F_{(1,32)}$ = 35.52; p<0.0001) but no main effect of the drug or an interaction effect (Fig 13B). Post hoc analysis revealed that both EX-SAL (adjusted p=0.0007) and EX-DSP4 (adjusted p=0.002) spent significantly more time grooming compared to CON-SAL mice (Fig. 13B). Further, EX-DSP4 mice spent significantly more time grooming compared to CON-DSP4 (adjusted p=0.002; Fig. 13B). There was no main effect of exercise or drug or interaction

effect for number of entries into the center of the testing arena (Fig. 13C) or the amount of time spent in the center of the arena (Fig. 13D).

5-10 minutes: During the second five-minute block of the open field task, we observed a main effect of exercise ($F_{(1,32)}$ =13.32; p=0.0009) but no main effect of drug or interaction effect on total distance moved (Fig. 14A). Post hoc analysis revealed significantly less distance traveled in EX-SAL (adjusted p=0.03) and EX-DSP4 (adjusted p=0.02) compared to CON-SAL mice (Fig. 14A). There was no significant difference between CON-DSP4 and EX-DSP4 mice. There was a main effect of exercise ($F_{(1,31)}$ = 28.31; p<0.0001) on total time spent grooming but no main effect of drug or interaction effect during the second five minute block (Fig. 14B). Post hoc analysis revealed that EX-DSP4 mice spent significantly more time grooming than CON-SAL (adjusted p=0.0006) and CON-DSP4 (adjusted p=0.0001) mice (Fig. 14B). There was no main effect of exercise or drug or an interaction effect for frequency of entries (Fig. 14C) or time spent (Fig. 14D) in the center of the testing arena.

10-15 minutes: During the final five-minute block of the open field task, we observed a main effect of exercise ($F_{(1,32)}$ = 10.55; p=0.003) and a main effect of the drug ($F_{(1,32)}$ = 5.091; p=0.03) but no interaction between exercise and drug for total distance traveled (Fig. 15A). Post hoc analysis revealed that EX-SAL (adjusted p=0.006) and EX-DSP4 (adjusted p=0.003) had significantly less distance traveled compared to CON-SAL (Fig 15A). Moreover, CON-DSP4 had significantly less distance traveled compared to CON-SAL (adjusted p=0.02; Fig

15A). We observed a main effect of exercise on time spent grooming ($F_{(1,31)}$ = 14.95; p=0.0005) but no main effect of drug or interaction (Fig 15B). Post hoc analysis revealed that EX-DSP4 mice spent significantly more time grooming than CON-SAL (adjusted p=0.01) and CON-DSP4 (adjusted p=0.002) mice (Fig 15B). We observed a main effect of exercise in the frequency of entries ($F_{(1,32)}$ = 6.869; p=0.01) and time spent ($F_{(1,32)}$ = 5.393; p=0.03) in the center of the testing arena (Fig 15C).

Running performance: We observed no significant difference between EX-SAL and EX-DSP4 in running performance, indicated by number of stimulus grid touches. However, we did observe a significant negative correlation between the total number of stimulus grid touches and distance traveled during the first five-minute block of the open field task (p=0.005). Better running performance was associated with higher activity in the open field task during the first five-minute block. This correlation was no longer observed during the second and third five-minute blocks. There was no correlation between running performance and time spent self-grooming or entries into the center of the testing arena during any five-minute time block.

Figure 7. Intraperitoneal injection of epinephrine induces GluR1 Ser845 phosphorylation in the hippocampus. A) Blots for p-Ser845, p-Ser831, and total GluR1, 15 minutes following saline (n=3) or epinephrine (n=4) IP injections. B) IP injection of epinephrine increased the ratio of Ser845 phosphorylation over GluR1 protein expression ($p=0.03$). There was no influence of epinephrine on the ratio of Ser831 phosphorylation over GluR1 or the ratio of GluR1 over NeuN. Error bars represent SEM. * indicates $p<0.05$

Figure 8. Acute exercise does not influence GluR1 phosphorylation in the mouse hippocampus. Mice were exposed to 30 minutes (after 6 minute warm up) of moderate-intensity (12m/min; n=11), high-intensity (15-17m/min; n=11), or no exercise treadmill exposure (n=11). Mice were sacrificed and hippocampi isolated immediately after treadmill exposure. Whole hippocampal homogenates were sonicated and boiled in 1% SDS. There was no significant effect of acute exercise on GluR1 phosphorylation. Error bars represent SEM.

Figure 9. Acute exercise does not influence glutamate receptor subunit mRNA expression in the mouse hippocampus. Mice were exposed to 30 minutes (after 6 minute warm up) of moderate-intensity (12m/min; n=11), high-intensity (15-17m/min; n=12), or no exercise treadmill exposure (n=12) Mice were sacrificed and hippocampi immediately isolated. mRNA was isolated from whole hippocampal homogenates in Trizol. Target mRNA expression is presented as $2^{-\Delta\Delta Ct}$ relative to the geometric mean of *ActB* and *Gapdh*. qPCR

analysis indicated that there was no significant effect of acute exercise on *GluR1*, *NR2A*, or *NR2B* mRNA expression. Error bars represent SEM.

Figure 10. High-intensity exercise increases transcript-specific Bdnf expression. Mice were exposed to 30 minutes (after 6 minute warm up) of moderate-intensity (12m/min; n=12), high-intensity (15-18 m/min; n=12), or no exercise treadmill (n=12). Mice were sacrificed and hippocampi immediately isolated. mRNA was isolated from whole hippocampal homogenates in Trizol. Target mRNA expression is presented as $2^{-\Delta\Delta Ct}$ relative to the geometric mean of *ActB* and *Gapdh.* A) A significant effect of exercise on *Bdnf IV* mRNA expression was observed ($F_{(2,\ 32)}$=3.79; p=0.03), after an acute bout of high-intensity exercise relative to controls (p=0.03). B) We found no effect of exercise on total *Bdnf* mRNA expression. Error bars represent SEM. * indicates p<0.05

Figure 11. Acute exercise does not influence time spent with objects or object location memory. Mice were exposed to 30 minutes (after 6 minute warm up) to high-intensity (15-17 m/min; n=15) or no exercise treadmill (n=15) and immediately underwent the novel object placement task. A) There was no main effect of acute exercise, test phase, or interaction in the time spent exploring the two objects. B) There was no effect of acute exercise on % time spent exploring the moved object relative to the time spent exploring both objects. Error bars represent SEM.

Figure 12. High-intensity acute exercise reduces exploratory behavior in the object location task. Mice were exposed to 30 minutes (after 6 minute

warm up) to high intensity (15-17 m/min; n=15) or no exercise treadmill (n=15) and immediately underwent the novel object location task. A) There was a main effect of acute exercise on total distance traveled ($F_{(1,28)}$=14.06; p=0.008) with mice exposed to high intensity treadmill running having significantly less distance traveled during the familiarization phase compared to treadmill controls (p=0.0003). B) There was a main effect of acute exercise ($F_{(1,28)}$=4.553; p=0.04) and an interaction between acute exercise and test phase ($F_{(1,28)}$=6.938; p=0.01) in number of interactions with the objects. Mice exposed to high-intensity treadmill running had significantly fewer interactions (# of interactions) with the objects during the familiarization phase relative to the treadmill controls (p=0.003). Error bars represent SEM. * indicates significantly different from control (p<0.05)

Figure 13. Open Field Task 0-5 Minutes. High intensity acute exercise induces anxiety-like behavior in the open field task during the first five minutes of the task. Saline-injected and DSP-4-injected mice were exposed to 30 minutes (after 6 minute warm up) of high intensity (15-17 m/min; EX-SAL n=8, EX-DSP4 n=9) or no exercise treadmill exposure (CON-SAL n=9, CON-DSP4 n=10) and immediately underwent the open field task. A) Mice exposed to high-intensity treadmill running had significantly less activity measured as total distance traveled ($F_{(1,32)}$ = 33.09; p<0.0001). x's indicate mice with ≥ 15 stimulus grid touches. B) Mice exposed to high-intensity treadmill running spent significantly more time self-grooming compared to no exercise treadmill controls ($F_{(1,32)}$ = 35.52; p<0.0001). C) There was no significant effect of exercise or drug on number of entries into the center of the testing arena. D) There was no

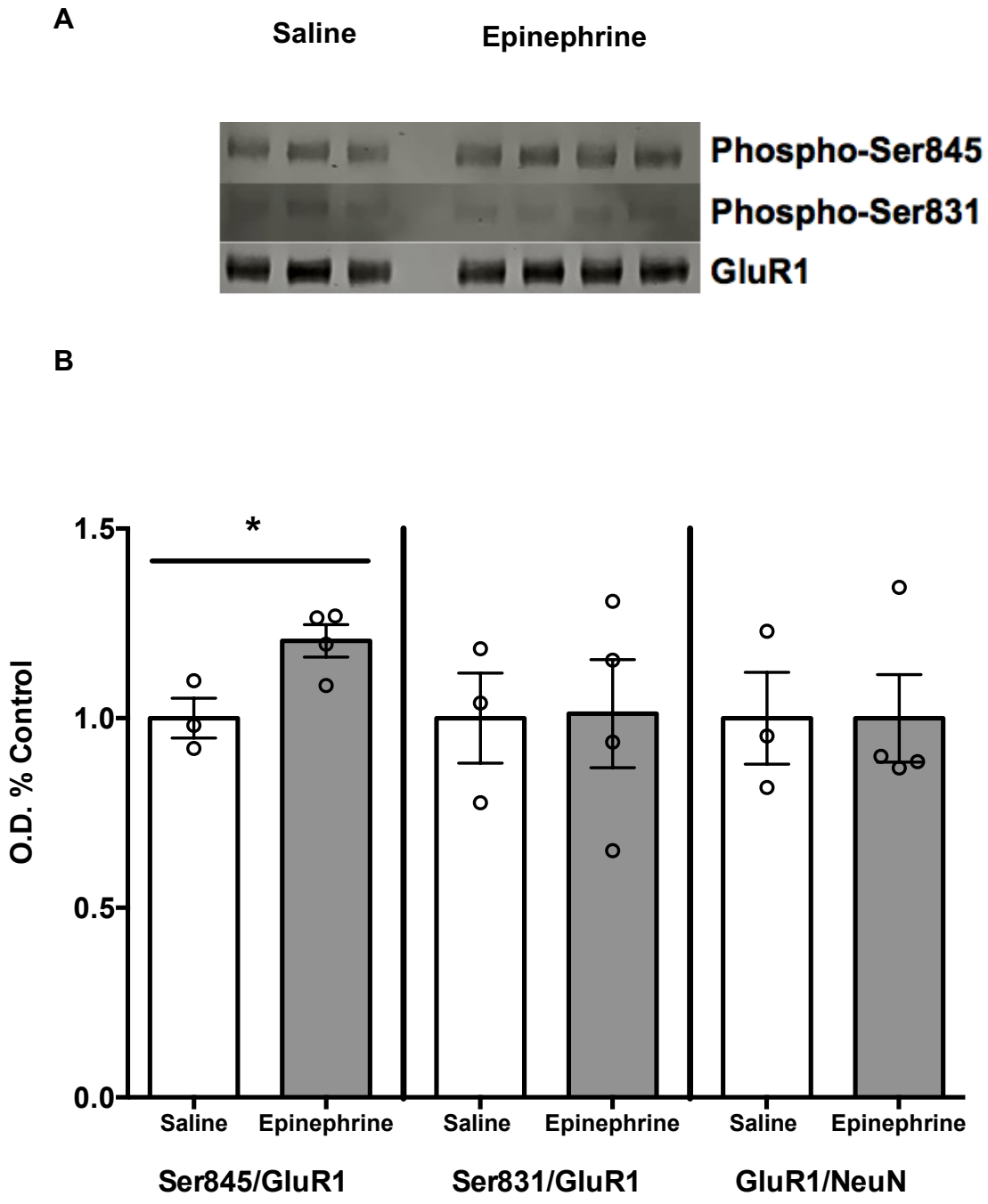
significant effect of exercise or drug on time spent in the center of the testing arena. Error bars represent SEM. * indicates significantly different from CON-SAL ($p<0.05$). $ indicates significantly different from CON-DSP4 ($p<0.05$)

Figure 14. Open Field Task 5-10 Minutes. High-intensity acute exercise induces anxiety-like behavior in the open field task during the second five-minute block (5-10 min) of the task. Saline-injected and DSP-4-injected mice were exposed to 30 minutes (after 6 minute warm up) of high-intensity (15-17 m/min; EX-SAL n=8, EX-DSP4 n=9) or no exercise treadmill exposure (CON-SAL n=9, CON-DSP4 n=10) and immediately underwent the open field task. A) Mice exposed to high-intensity treadmill running had significantly less activity measured as total distance traveled ($F_{(1,32)}$ =13.32; p=0.0009). x's indicate mice with ≥ 15 stimulus grid touches. B) Mice exposed to high-intensity treadmill running spent significantly more time self-grooming compared to no-exercise treadmill controls ($F_{(1,31)}$ = 28.31; $p<0.0001$). C) There was no significant effect of exercise or drug on number of entries into the center of the testing arena. D) There was no significant effect of exercise or drug on time spent in the center of the testing arena. Error bars represent SEM. * indicates significantly different from CON-SAL ($p<0.05$). $ indicates significantly different from CON-DSP4 ($p<0.05$)

Figure 15. Open Field Task 10-15 Minutes. High-intensity acute exercise induces anxiety-like behavior in the open field task during the third five-minute block (10-15 min) of the task. Saline-injected and DSP-4-injected mice

were exposed to 30 minutes (after 6 minute warm up) of high-intensity (15-17 m/min; EX-SAL n=8, EX-DSP4 n=9) or no exercise treadmill exposure (CON-SAL n=9, CON-DSP4 n=10) and immediately underwent the open field task. A) Mice exposed to high-intensity treadmill running had significantly less activity measured as total distance traveled ($F_{(1,32)}$ = 10.55; p=0.003). Mice injected with DSP-4 had significantly less activity measured as total distance traveled ($F_{(1,32)}$ = 5.091; p=0.03). x's indicate mice with ≥ 15 stimulus grid touches. B) Mice exposed to high-intensity treadmill running spent significantly more time self-grooming compared to no-exercise treadmill controls ($F_{(1,31)}$ = 14.95; p=0.0005). C) Mice exposed to high-intensity treadmill running had significantly fewer entries into the center of the testing arena compared to no-exercise treadmill controls ($F_{(1,32)}$ = 6.869; p=0.01). D) Mice exposed to high-intensity treadmill running spent significantly less time in the center of the testing arena compared to no-exercise treadmill controls ($F_{(1,32)}$ = 5.393; p=0.03). Error bars represent SEM. * indicates significantly different from CON-SAL ($p<0.05$). $ indicates significantly different from CON-DSP4 ($p<0.05$)

Figure 7.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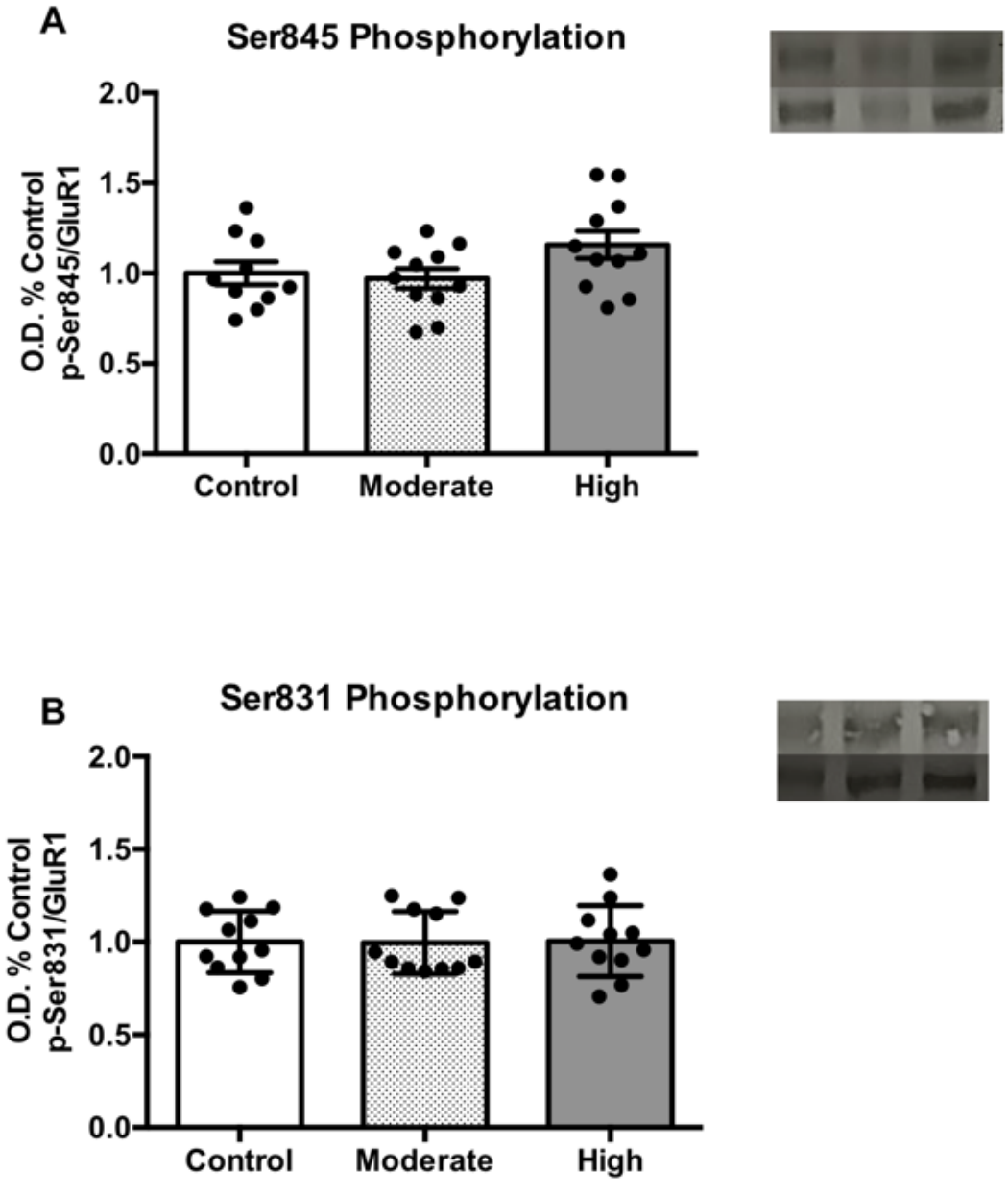
Saline
Epinephrine
Phospho-Ser845
Phospho-Ser831
GluR1
B
O.D. % Control
1.5
1.0
0.5
0.0
*
Saline
Epinephrine
Saline
Epinephrine
Saline
Epinephrine
Ser845/GluR1
Ser831/GluR1
GluR1/NeuN

Figure 8.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O.D. % Control
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1.5
1.0
0.5
0.0
Control
Moderate
High
B
Ser831 Phosphorylation
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p-Ser831/GluR1
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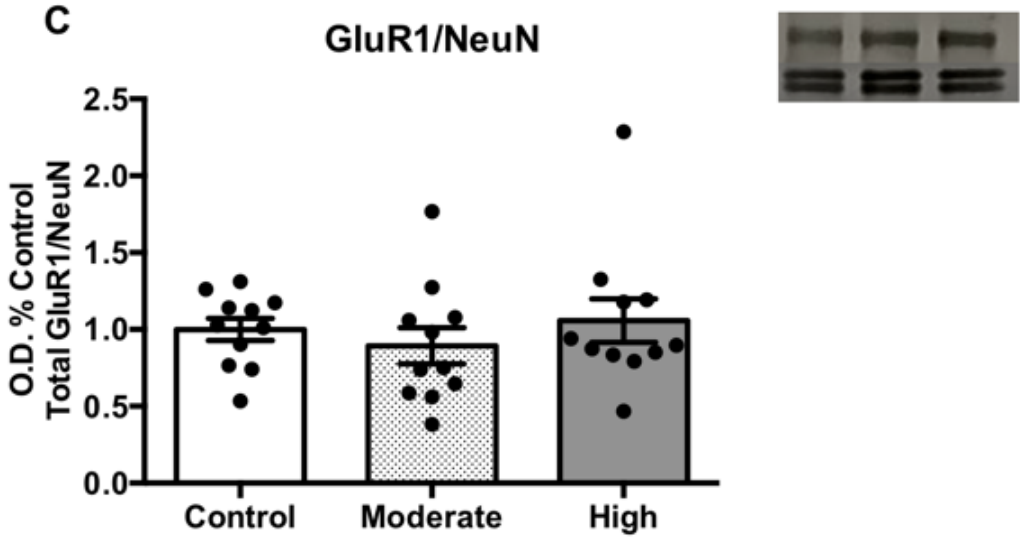
1.5
1.0
0.5
0.0
Control
Moderate
High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GluR1/NeuN
O.D. % Control
Total GluR1/NeuN
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2.0
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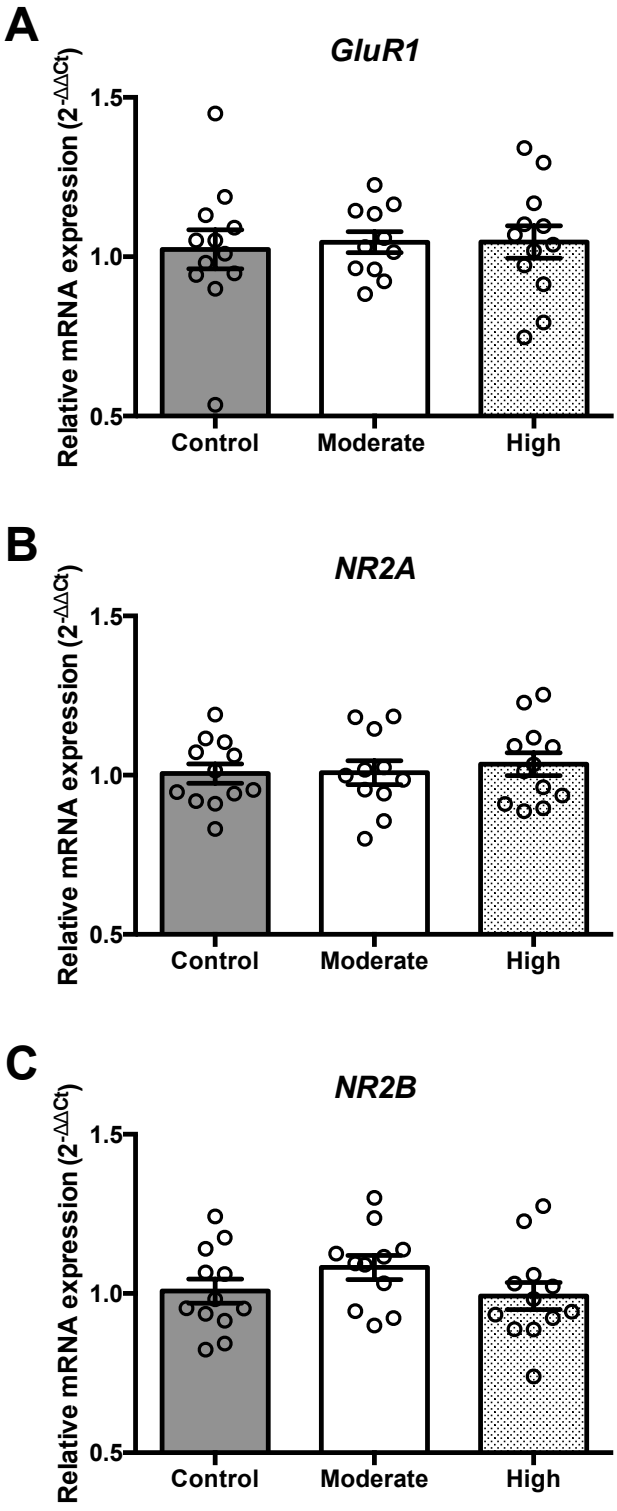
1.0
0.5
0.0
Control
Moderate
High

Figure 9.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Relative mRNA expression (2-ΔΔCt)
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1.0
0.5
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Moderate
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NR2B
Relative mRNA expression (2-ΔΔCt)
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1.0
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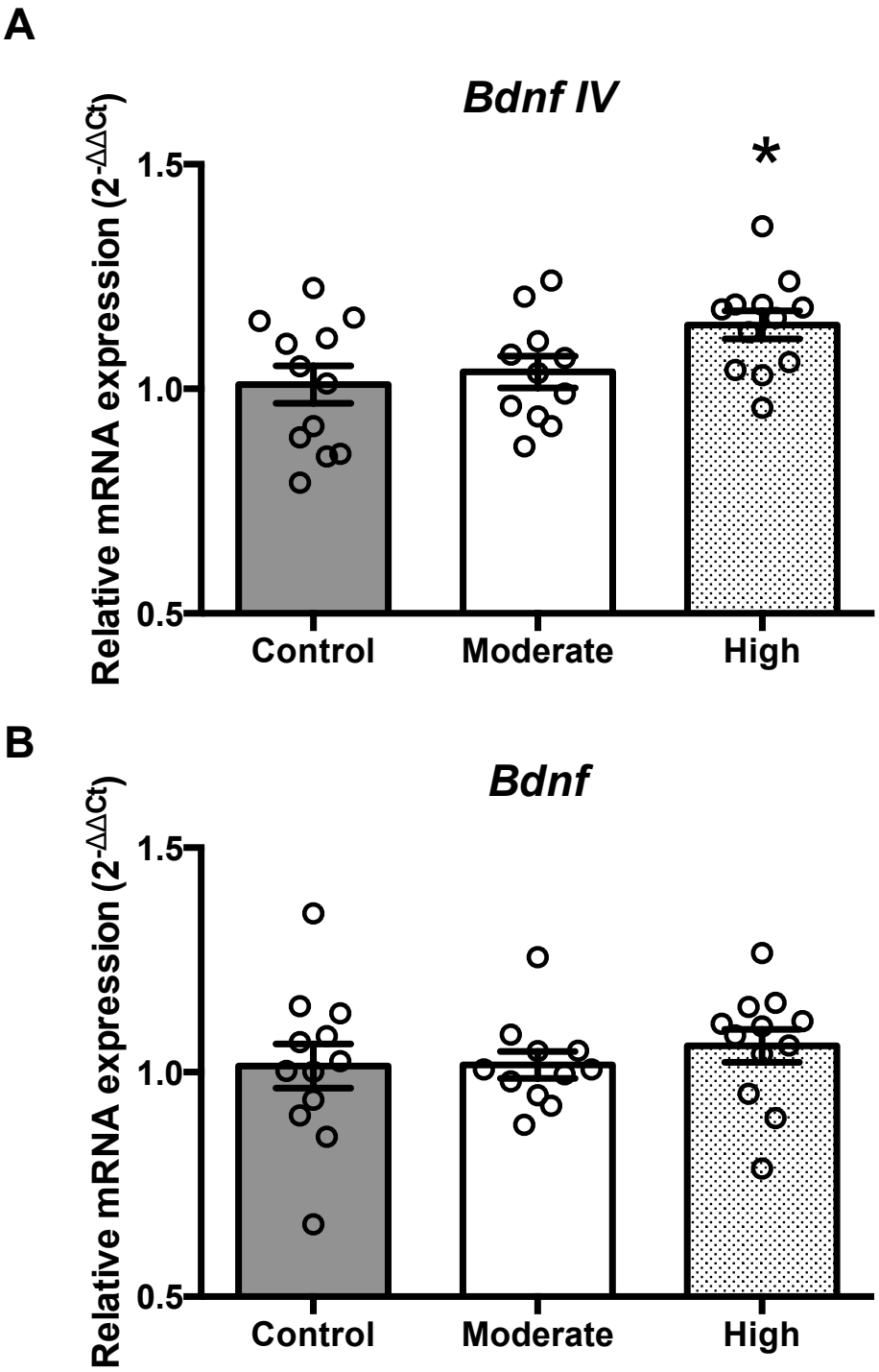
Control
Moderate
High

Figure 10.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Relative mRNA expression (2-ΔΔCt)
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1.0
0.5
*
Control
Moderate
High
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Bdnf
Relative mRNA expression (2-ΔΔCt)
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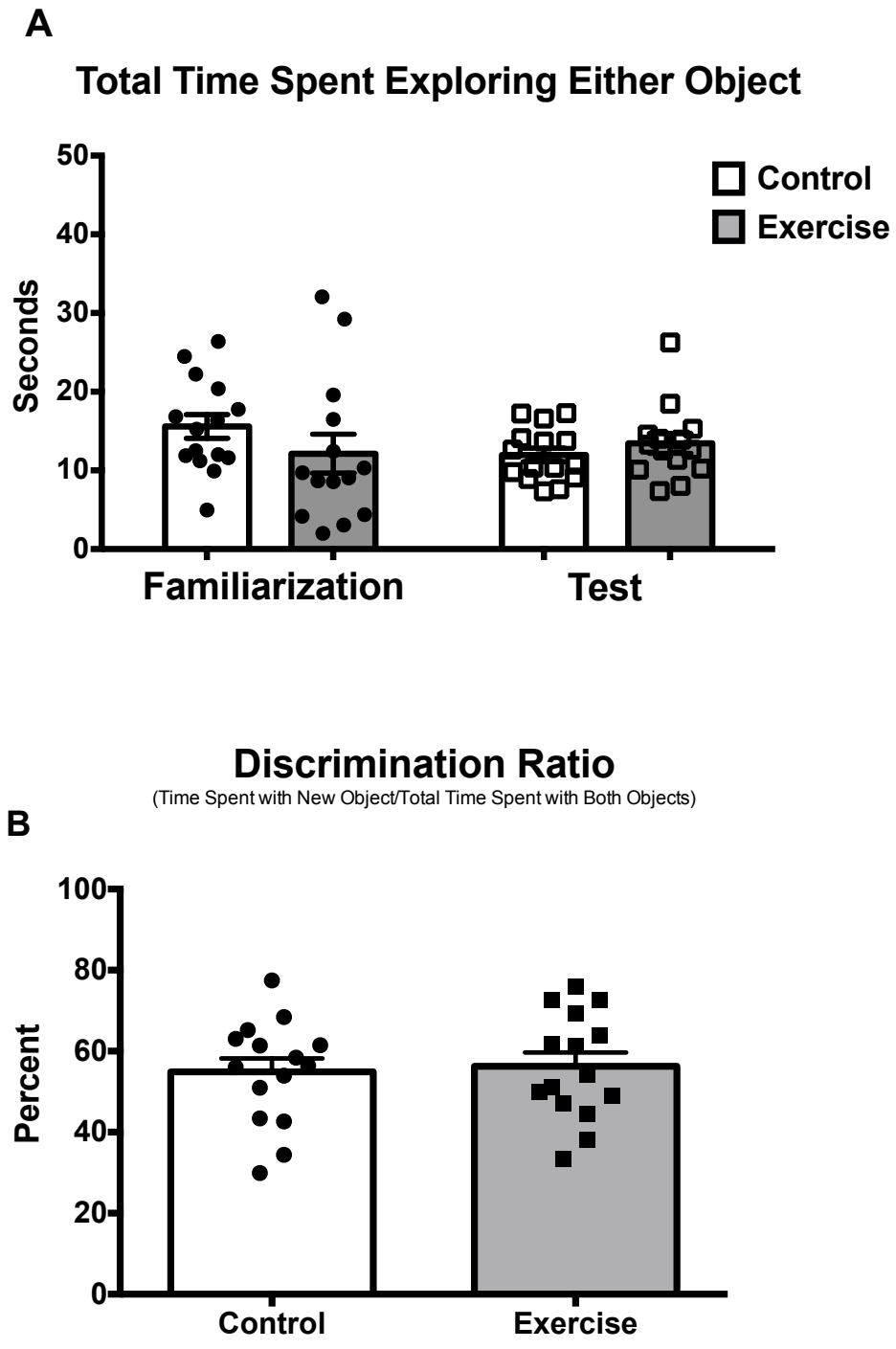
1.0
0.5
Control
Moderate
High

Figure 11.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Exercise
Seconds
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40
30
20
10
0
Familiarization
Test
Discrimination Ratio
(Time Spent with New Object/Total Time Spent with Both Objects)
B
Percent
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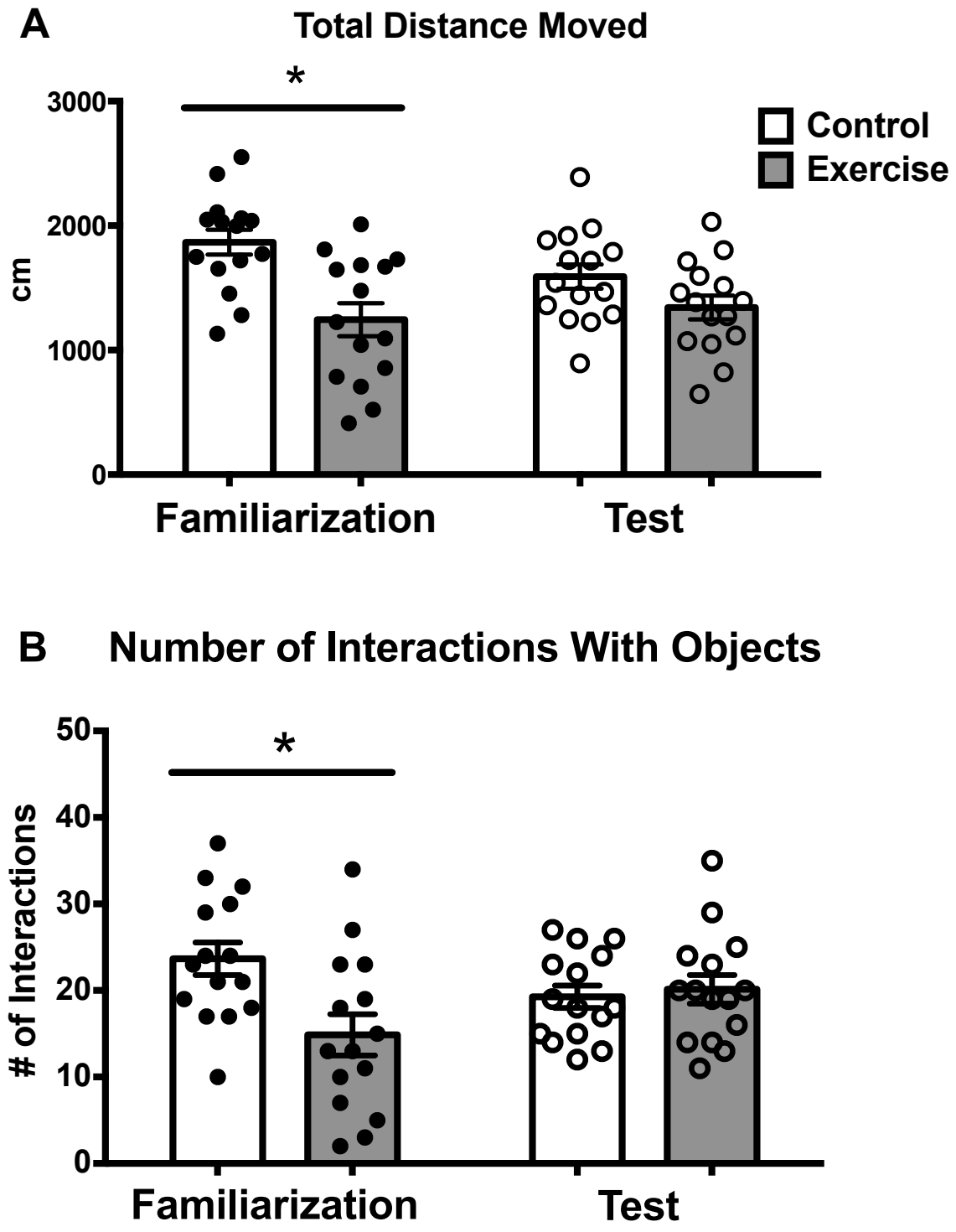
80
60
40
20
0
Control
Exercise

Figure 12.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2000
1000
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cm
Control
Exercise
Familiarization
Test
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Number of Interactions With Objects
*
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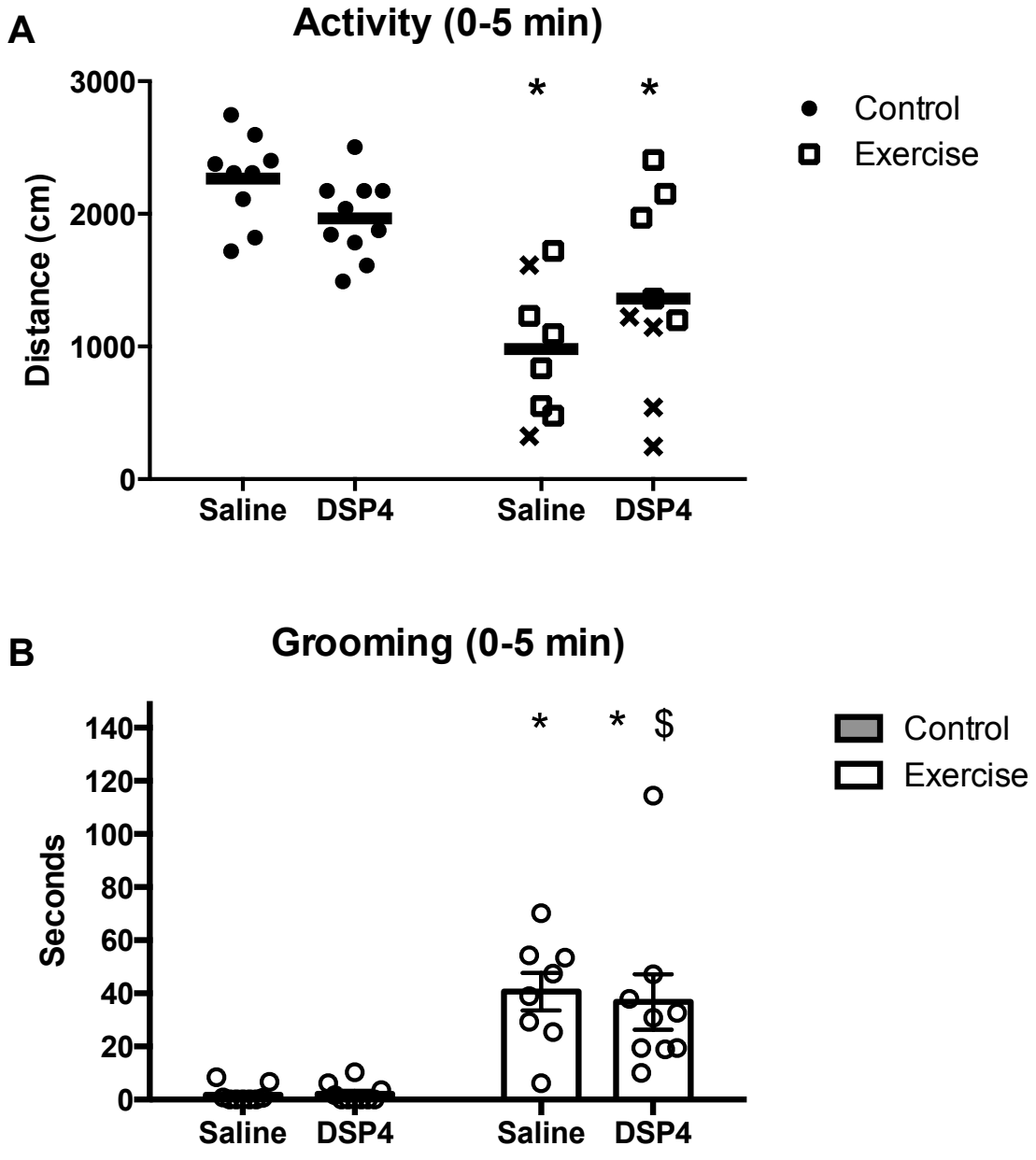
20
10
0
# of Interactions
Familiarization
Test

Figure 13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Distance (cm)
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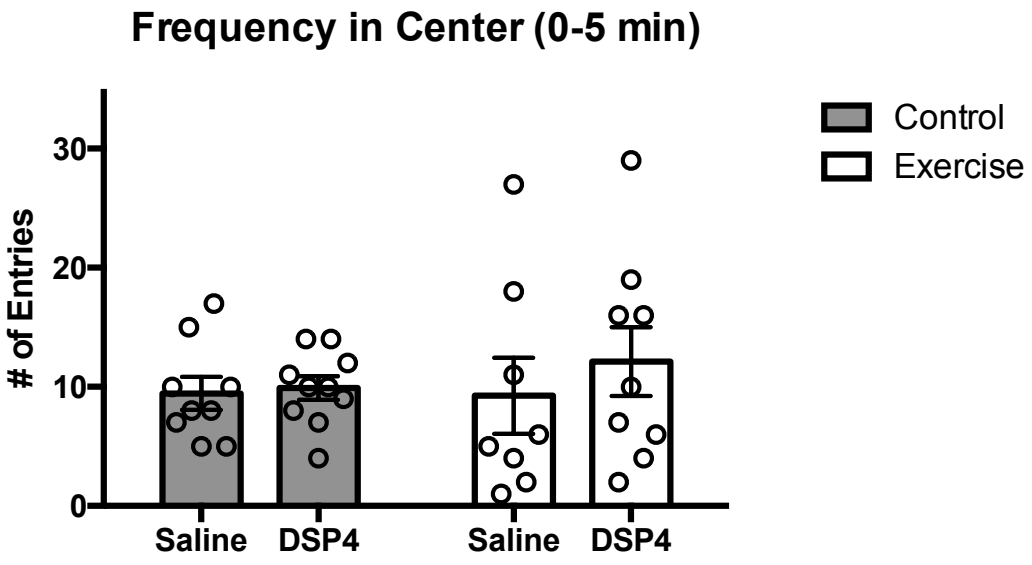
2000
1000
0
Saline
DSP4
Saline
DSP4
Control
Exercise
B
Grooming (0-5 min)
Seconds
140
120
100
80
60
40
20
0
Saline
DSP4
Saline
DSP4
Control
Exercise

C


Frequency in Center (0-5 min)
# of Entries
30
20
10
0
Saline
DSP4
Saline
DSP4
Control
Exercise

D

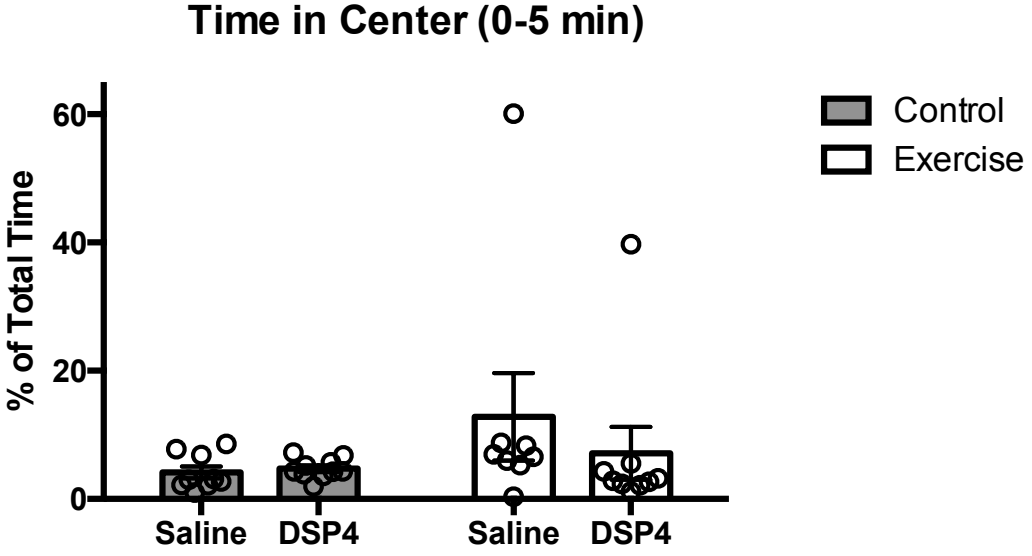
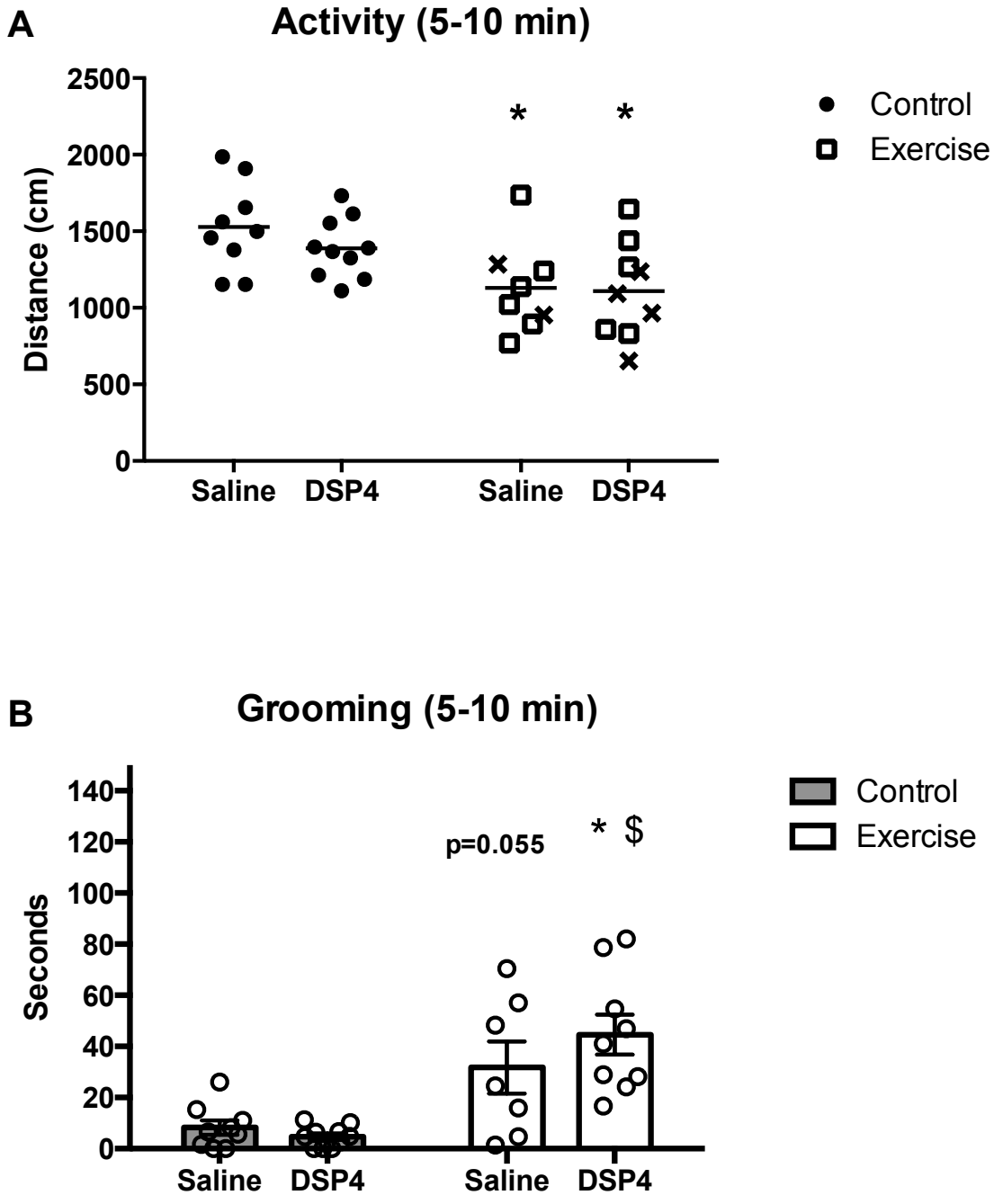
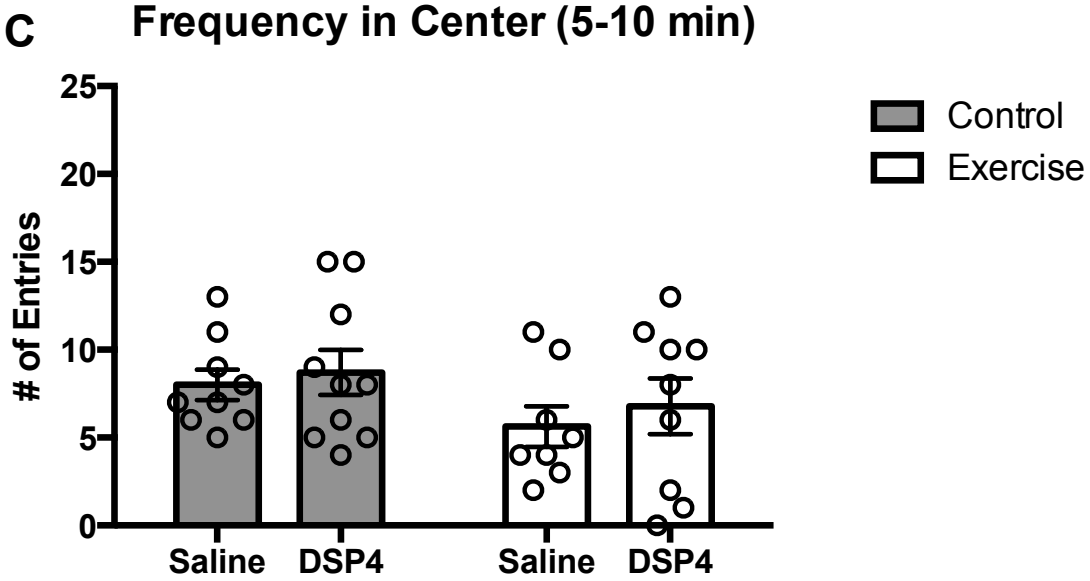

Time in Center (0-5 min)
% of Total Time
60
40
20
0
Saline
DSP4
Saline
DSP4
Control
Exercise

Figure 14.


A
Activity (5-10 min)
Distance (cm)
2500
2000
1500
1000
500
0
Saline
DSP4
Saline
DSP4
*
*
Control
Exercise
B
Grooming (5-10 min)
Seconds
140
120
100
80
60
40
20
0
p=0.055
* $
Saline
DSP4
Saline
DSP4
Control
Exercise


C
Frequency in Center (5-10 min)
# of Entries
25
20
15
10
5
0
Saline
DSP4
Saline
DSP4
Control
Exercise

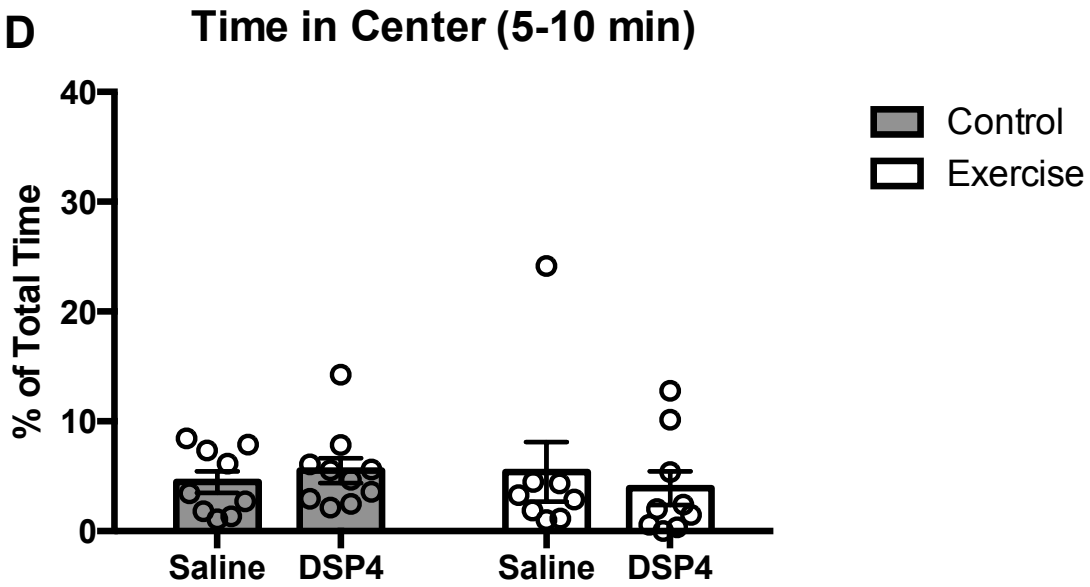
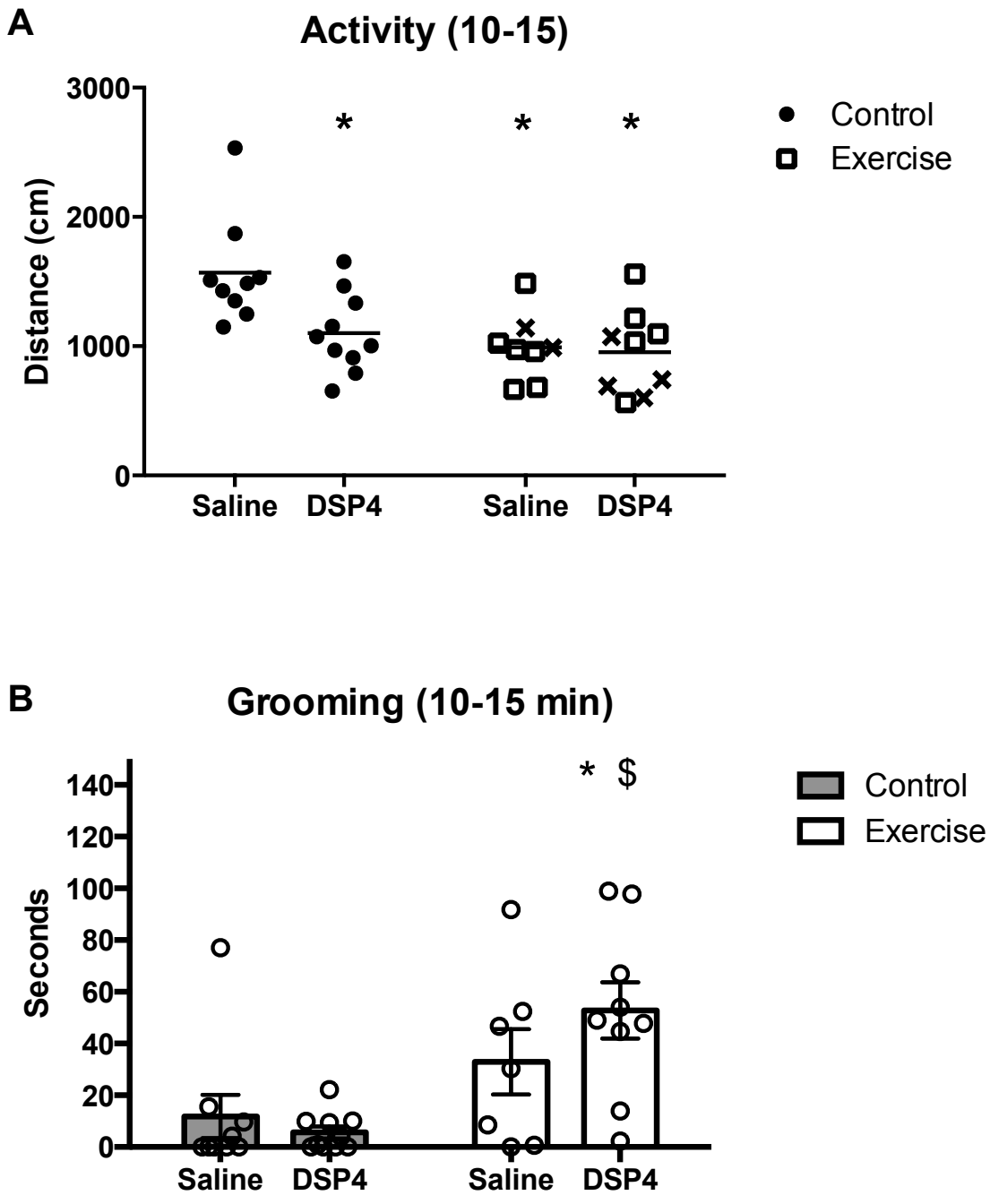
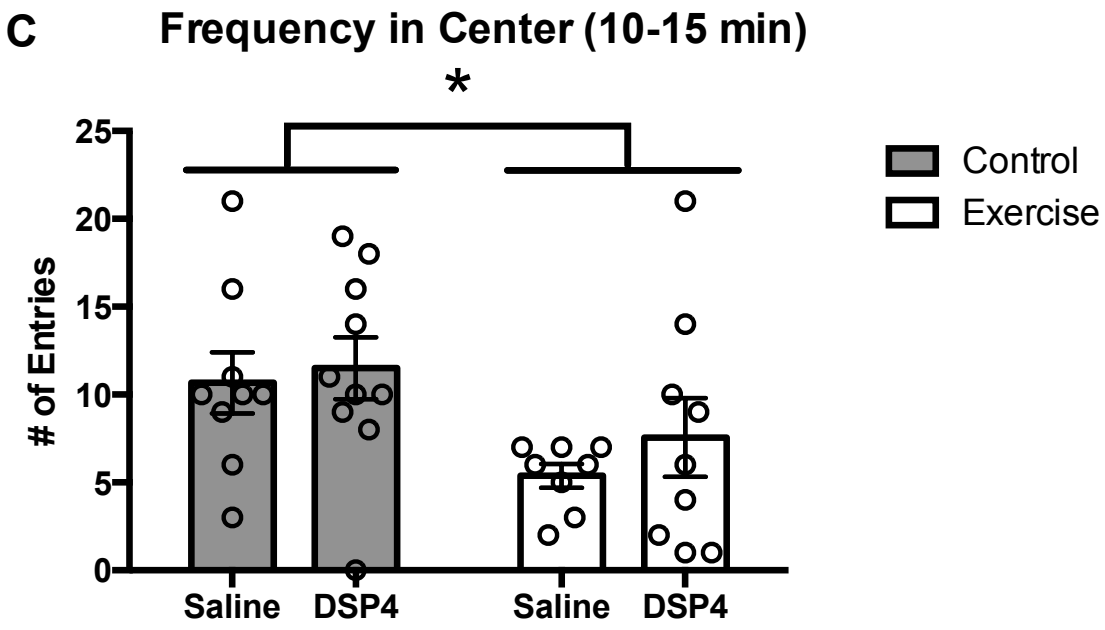
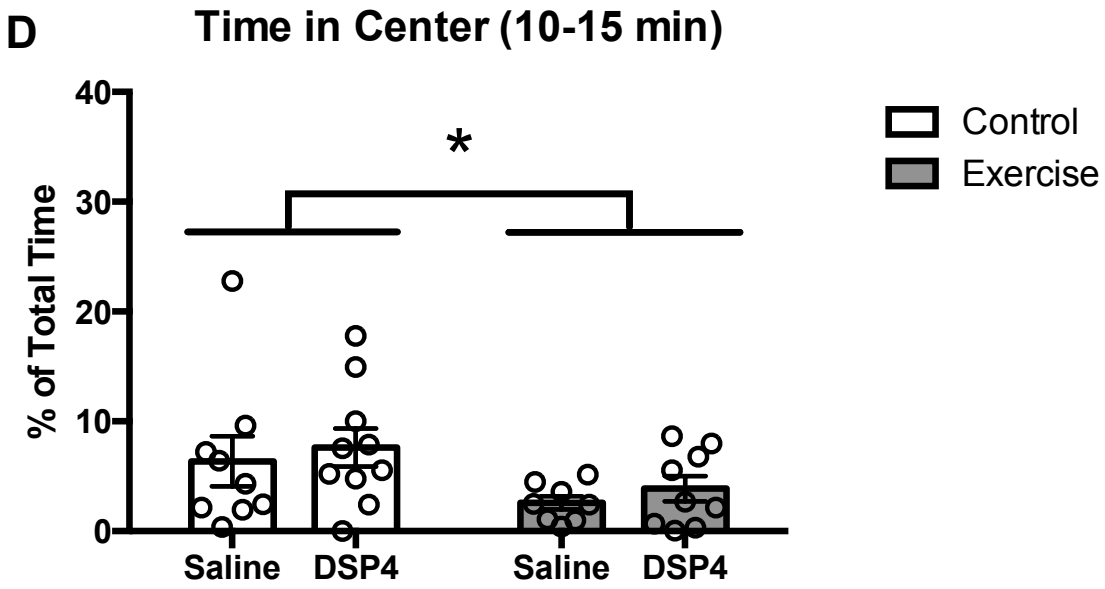

D
Time in Center (5-10 min)
% of Total Time
40
30
20
10
0
Saline
DSP4
Saline
DSP4
Control
Exercise

Figure 15.


A
Activity (10-15)
Distance (cm)
3000
2000
1000
0
Saline
DSP4
Saline
DSP4
Control
Exercise
B
Grooming (10-15 min)
Seconds
140
120
100
80
60
40
20
0
Saline
DSP4
Saline
DSP4
Control
Exercise


C
Frequency in Center (10-15 min)
*
# of Entries
25
20
15
10
5
0
Saline
DSP4
Saline
DSP4
Control
Exercise


D
Time in Center (10-15 min)
*
% of Total Time
40
30
20
10
0
Saline
DSP4
Saline
DSP4
Control
Exercise

Discussion

We found that a single 30-minute bout of high intensity treadmill exercise was sufficient to increase mRNA expression of *Bdnf* transcript IV; however, unlike acute psychological stress (Hu *et al.*, 2007) or peripheral catecholamines, one 30-minute bout of acute exercise did not influence the phosphorylation status of the GluR1 subunit of the AMPAR. To determine if acute exercise improves memory performance in mice, an effect observed in humans (Lambourne & Tomporowski, 2010; Roig *et al.*, 2013), we exposed mice to a low stress, one-trial memory task immediately following the acute bout of exercise. We observed that mice exposed to the acute bout of exercise spent less time exploring the environment and interacting with the objects to be remembered. Therefore, we tested if mice exposed to acute exercise displayed anxiety-like behavior in the open field task and if the behavior associated with acute exercise was influenced by central noradrenergic signaling. We found that acute exercise significantly reduced locomotor activity and significantly increased time spent self-grooming, common indicators of an anxious phenotype. Moreover, our data suggest that LC-noradrenergic signaling may influence behavior following treadmill exposure.

We were able to replicate previous findings that an IP injection of epinephrine increases phosphorylation of Ser845 of GluR1 but did not influence phosphorylation of Ser831 (Hu *et al.*, 2007). We hypothesized that, like acute psychological stress and peripheral injections of epinephrine (Hu *et al.*, 2007), acute forced treadmill exercise would increase phosphorylation of GluR1 at

Ser845 in the hippocampus, potentially via the release of catecholamines and central noradrenergic signaling. However, 30 minutes of high- or moderate-intensity acute exercise was insufficient to induce Ser845 phosphorylation. While there is evidence of short-term physical activity increasing phosphorylation of Ser845 in the rodent cortex (Mizutani *et al.*, 2015), this has not been investigated following a single bout of acute exercise. Our data suggest that a single acute bout of exercise does not induce phosphorylation of Ser845 on GluR1.

Potentially, we did not observe an effect of the acute exercise on GluR1 phosphorylation because 30 minutes of exercise was not sufficient to elevate peripheral epinephrine or central norepinephrine. Pagliari and Peyrin (1995a) found that cortical norepinephrine did not increase in response to treadmill running until after ~40 minutes of exercise in the rat while Goekint et al. (2012) observed no influence of 60 minutes of treadmill running on extracellular hippocampal norepinephrine. Interestingly, Goekint et al. (2012) observed a significant effect of exercise on dopamine release, another neuromodulator that can increase GluR1 Ser845 phosphorylation via PKA signaling (Carr *et al.*, 2010). In contrast to these experiments, Dishman et al. (2000) reported that 15 minutes of treadmill running or immobilization stress decreased norepinephrine levels in the LC and hippocampus, likely through release and metabolism of the neurotransmitter. Potentially, longer more exhausting exercise would result in an increase in GluR1 phosphorylation. Alternatively, it is possible that 30 minutes of exercise was sufficient to elevate central norepinephrine, though sacrificing the mice immediately after the bout of exercise did not provide enough time for

intracellular signaling necessary for phosphorylation of GluR1. In our epinephrine injection experiment, we waited 15 minutes post-injection before sacrifice, similar to the procedure of Hu et al. (2007). It is possible that we would have observed an effect if we waited longer between the cessation of exercise and sacrifice of the animals.

Exposure to the treadmill environment alone may have been sufficient to elevate Ser845 phosphorylation, which might have masked the effect of acute exercise. The three days of acclimation to the treadmill environment and the 36-minute exposure to the treadmill even in the stationary controls likely produced novelty-induced arousal which may have activated the noradrenergic system and stimulated hippocampal β_2 adrenergic receptors (King & Williams, 2009). Indeed, novelty exploration increases neuronal activity in the LC and release of norepinephrine in the hippocampus (Sara *et al.*, 1994). Without a cage-control group that did not undergo the acclimation or experimental day treadmill exposure, we are unable to determine if treadmill exposure alone increased phosphorylation of the GluR1 subunit of the AMPA receptor.

In addition to Ser845, it is curious that we did not observe an effect of exercise on Ser831 phosphorylation. Though we hypothesized that elevated noradrenergic signaling due to the intense forced exercise would induce Ser845 phosphorylation, Ser831 is phosphorylated by CAMKII (Barria *et al.*, 1997), a kinase that has been linked to exercise-induced hippocampal plasticity (Voss *et al.*, 2013). Once again, it is possible that exposure to the novel environment of

the treadmill even in controls was sufficient to elevate CAMKII activity and subsequent phosphorylation of Ser831, occluding any additional benefit of the exercise exposure.

There was no effect of acute exercise on mRNA expression of *GluR1*, *NR2A*, or *NR2B*. It is likely that the short time between the start of exercise and sacrifice was insufficient for activity-induced transcription of glutamate receptors. The literature reporting the effects of exercise training on glutamate receptor expression is inconsistent. Previous research has reported an increase in NR2B mRNA expression following short-term exposure to a voluntary running wheel (Molteni *et al.*, 2002; Farmer *et al.*, 2004). Higher expression of NR2B relative to NR2A is associated with a more plastic synapse (Tang *et al.*, 1999; Cao *et al.*, 2007), making this a potential mechanism for exercise-induced plasticity and reduced threshold for LTP and learning. Molteni et al. (2002) reported that three days of voluntary running, a protocol generally considered acute exercise, increased both NR2B, NR2A, and to a much lesser extent, GluR1 expression in the rat hippocampus. NR2A remained significantly different than controls after seven days of wheel running but was no longer significantly different after one-month of exposure. Ni et al. (2009) found that GluR1 mRNA expression was not influenced by six days of daily treadmill running in healthy Sprague-Dawley rats. Our data demonstrate that an acute bout of treadmill exercise does not stimulate rapid transcription of glutamate receptor subunits in the mouse hippocampus and multi-day exposures or voluntary wheel running may be necessary to increase mRNA expression of these subunits.

Brain-Derived Neurotrophic Factor. We observed a significant effect of acute exercise and acute exercise intensity on *Bdnf IV* mRNA expression but observed no effect on total *Bdnf*. Numerous studies have demonstrated that exercise increases protein and mRNA expression of Bdnf in the rodent hippocampus (Neeper *et al.*, 1996; Molteni *et al.*, 2002; Vaynman *et al.*, 2003; 2004; Berchtold *et al.*, 2005; Ding *et al.*, 2011; Sartori *et al.*, 2011; Venezia *et al.*, 2016). To our knowledge, this is the first investigation to report that one acute bout of exercise can increase expression of *Bdnf IV*. The effect was only observed in mice that were exposed to high-intensity treadmill running, which likely caused high neuronal activity in the hippocampus that resulted in rapid transcription of the activity-dependent *Bdnf* transcript IV. Bdnf is important for hippocampal neurogenesis (Lee *et al.*, 2002; Sairanen, 2005; Scharfman *et al.*, 2005; Rossi *et al.*, 2006; Taliaz *et al.*, 2009), synaptic plasticity (Korte *et al.*, 1995; Figurov *et al.*, 1996; Korte *et al.*, 1996; Patterson *et al.*, 1996; Kang *et al.*, 1997; Ma *et al.*, 1998; Chen *et al.*, 1999; Zakharenko *et al.*, 2003), and memory (Linnarsson *et al.*, 1997; Ma *et al.*, 1998; Mu *et al.*, 1999; Mizuno *et al.*, 2000; Alonso *et al.*, 2002; Heldt *et al.*, 2007; Bekinschtein *et al.*, 2008), all of which are associated with exercise training (Cotman *et al.*, 2007; Vivar *et al.*, 2012; Voss *et al.*, 2013). In fact, blocking Bdnf action with an antibody that binds to its receptor prevents the exercise-induced improvement in spatial memory and expression of plasticity-associated genes (Vaynman *et al.*, 2004). The finding that an acute bout of exercise of only 30 minutes influenced transcription of the *Bdnf* gene is

interesting and suggests that the process of hippocampal plasticity begins with short exposures to exercise, albeit forced and highly stressful in our investigation.

Acute exercise caused an increase in *Bdnf IV* but not total *Bdnf* expression, suggesting that *Bdnf IV* transcription is rapidly initiated in response to exercise, similar to an immediate early gene, while the other *Bdnf* transcripts have a slower pattern of transcription. *Bdnf IV* is highly sensitive to neuronal activity (Tao *et al.*, 1998; 2002; Martinowich *et al.*, 2003) and hippocampal expression increases in response acute immobilization stress (Marmigère *et al.*, 2003), fear conditioning (Lubin *et al.*, 2008), and exercise training (Zajac *et al.*, 2009; Intlekofer *et al.*, 2013). It is important to note that our exercise protocol was highly stressful as it was unpredictable and uncontrollable making it similar in certain ways to both fear conditioning and immobilization stress. In a fear conditioning task, context exposure alone increased total *Bdnf* expression through increased expression of *Bdnf I* and *VI*, though following associative fear conditioning, the increase in total *Bdnf* transcription was mediated by an increase in *Bdnf IV* transcription (Lubin *et al.*, 2008). Marmigere et al. (2003) reported that 15 and 60 minutes of acute immobilization stress increased *Bdnf IV* (III in the paper) in the rat hippocampus, demonstrating a rapid transcription of *Bdnf IV*, which was more rapid than total *Bdnf* mRNA and other *Bdnf* transcripts that were not influenced by immobilization stress until 60 minutes. This presents a possible explanation for why we observed significantly increased *Bdnf IV* mRNA after 30 minutes but no change in total *Bdnf* at this time point.

Though forced treadmill exercise is stressful because it is unpredictable and uncontrollable, both moderate-intensity and high-intensity treadmill running were similarly uncontrollable and unpredictable and we only observed a difference in *Bdnf IV* expression in the high-intensity group. This indicates that signaling processes unique to the high-intensity exercise are stimulating transcription of *Bdnf IV.* Concerning the influence of acute exercise on *Bdnf* expression, research has been inconsistent in both the research approach and reported findings. Oliff et al. (1998) reported that six hours of voluntary wheel running increased *Bdnf* mRNA in the rat hippocampus (hilus, CA1, and CA3) but had no effect on *Bdnf IV* expression. Importantly, these investigators acclimated mice to the running wheel for three nights followed by a 10-day washout period. Though they observed no difference in *Bdnf IV* mRNA expression after six or 12 hours of voluntary wheel running, they found that mice that underwent the three days of wheel acclimation but no acute wheel exposure 10 days later had significantly elevated *Bdnf IV* in all hippocampal regions examined and greater total *Bdnf* in CA1. This is interesting and demonstrates the lasting influence that acclimation protocols can have on hippocampal *Bdnf* expression. Rasmussen et al. (2009) reported significantly greater *Bdnf* mRNA in the hippocampus two and six hours post-treadmill running to exhaustion but not immediately after exercise. Similar to Oliff et al. (1998), Rasmussen et al. (2009) used an acclimation protocol that included running on the treadmill for multiple days before the acute treadmill running. These current data and previously published research show that *Bdnf IV* transcription is sensitive to acute exercise and is rapidly transcribed

upon exposure. Potentially, longer acute exercise exposures are necessary to significantly increase total Bdnf expression.

The novel object location task is a hippocampal-dependent memory task (Barker & Warburton, 2011). We hypothesized that an acute bout of high-intensity exercise would lower the threshold for memory formation and improve memory performance as has been shown with epinephrine injections (Hu *et al.*, 2007) and three weeks of voluntary wheel running (Intlekofer *et al.*, 2013). In the current investigation, mice exposed to high-intensity acute exercise showed significantly less exploratory behavior (frequency of interaction with objects and significantly less distance moved) during the familiarization phase of the task compared to treadmill controls. Therefore, the mice were not actively exploring the novel objects that were to be remembered. This behavior suggested an anxious phenotype in the mice exposed to treadmill running (Crawley, 1985). The relationship between exercise and anxiety in rodents is complex and the literature supports both anxiolytic and anxiogenic effects of exercise (for review, see Sciolino & Holmes, 2012). Potentially, elevated stress hormones induced by acute exercise, which we thought would improve memory, contributed to the anxiety-like behavior but returned to baseline by the test phase, when we no longer observed reduced exploratory behavior. Another potential explanation is that the mice were simply fatigued following the bout of treadmill exercise. This is unlikely because the treadmill exercise was not exhaustive, though even low levels of fatigue could have contributed to the observed behavior. The behavioral profile observed in the object location task following acute exercise was

consistent with observations in the open field task, where exercised mice showed significantly less activity during the task and spent significantly more time self-grooming. We also observed reduced entries and total time spent in the center of the testing arena in exercised mice during the final five minute time block. This is a hallmark measure of anxiety behavior in the open field task (Prut & Belzung, 2003). Self-grooming is a complex behavior that can be interpreted as a marker of anxiety since stressful situations and high levels of anxiety can increase grooming behavior (for review, see Kalueff *et al.*, 2015). Moreover, this behavior can be reduced with the use of benzodiazepines (Kalueff *et al.*, 2015). However, grooming behavior is mediated by many brain regions/circuits and can be influenced by numerous pharmacological manipulations (Kalueff *et al.*, 2015), so interpretation of this behavior following forced exercise is difficult. It could represent reduced vigilance and more internally directed behavior (Sothmann *et al.*, 1996). Taken together, these data suggest that acute exercise stimulates behaviors that may be interpreted as anxiogenic; however, more research is needed to determine if this is truly anxiety-like behavior or an effect of exercise on motivation or fatigue.

The majority of published research on exercise and anxiety has reported anxiolytic effects of exercise (Sciolino & Holmes, 2012); however, a few studies have reported anxiogenic effects (Fuss *et al.*, 2009; 2010; Onksen *et al.*, 2012). The investigations of exercise and anxiety have focused on chronic voluntary wheel running and the anxiogenic mechanism identified, increased neurogenesis, is unlikely to explain our findings since one 30-minute bout of exercise is not

sufficient to generate new functioning neurons (Van der Borght *et al.*, 2009). Salam et al. (2009) provided C57Bl/6J mice with access to a voluntary running wheel for two weeks prior to exposure to the open field task and found conflicting results. They reported that running-exposed mice spent significantly more time in the center of the testing box and entered the center more frequently than sedentary mice. These behaviors are indicative of less anxiety; however, they also observed significantly less activity and more grooming behavior in runners, which is indicative of higher levels of anxiety, similar to what we observed. Interestingly, Duman et al. (2008) reported that three weeks of voluntary wheel running in C57Bl/6J mice increased anxiety-like behavior (reduced activity) in the open field task if the task was initiated the morning after a night of voluntary wheel running. In contrast, they observed anxiolytic-like behavior if the task was initiated 24 hours after the last exposure to the voluntary running wheel. These data suggest a transient anxiogenic effect of exercise.

Potentially, our observed behavior resulted from elevated release of adrenal stress hormones and central noradrenergic signaling, which we predicted would improve learning and memory. The reduced locomotor behavior observed in the object location and open field tasks is similar to the home cage behavior we observed following an IP injection of epinephrine (unpublished observation). Administration of selective norepinephrine reuptake inhibitors used as antidepressants (e.g. reboxetine) have been shown to be initially anxiogenic (Inoue *et al.*, 2006), though become anxiolytic after chronic administration by reducing stress-induced cortical norepinephrine release (Dazzi *et al.*, 2003).

Potentially, acute and chronic exercise function similarly to acute and chronic treatment with selective norepinephrine reuptake inhibitors by increasing extrasynaptic norepinephrine, which is acutely anxiogenic but becomes anxiolytic with chronic exposure. Indeed the role of norepinephrine in anxiety is complex and is associated with both anxiolytic and anxiogenic behavior depending on the type of acute stress stimulating the norepinephrine release (for review, see Goddard *et al.*, 2010). We hypothesized that the anxiogenic-like behavior observed following acute exercise would be attenuated with pre-treatment with the selective neurotoxin for the LC- noradrenergic system, DSP-4. The influence of DSP-4 on behavior in the open field task was small. In contrast to our hypothesis, mice exposed to exercise and injected with DSP-4 had significantly reduced activity compared to control mice injected with saline; however, they did not have significantly reduced activity compared to control mice injected with DSP-4 at any time point. This suggests that DSP-4 attenuates the effect of acute exercise on exploratory behavior but does not rescue the effect of exercise completely. Further, mice injected with DSP-4 and exposed to exercise spent significantly more time self-grooming compared to both saline and DSP-4 injected treadmill control mice, and DSP-4 did not attenuate the effect of acute exercise on frequency of entries or time spent in the center of the arena in the last five minutes of the open field task. These data indicate that DSP-4 was ineffective at preventing the anxiety-like effect of acute forced treadmill exercise, though it did attenuate the effect of acute exercise on activity during the task.

DSP-4 treatment has been shown to influence open field behavior by reducing overall activity but this can be attenuated with chronic mild stress (Harro *et al.*, 1999). We did not observe a significant reduction in activity in animals that received DSP-4 alone, potentially due to the mild stressful nature of the novel treadmill environment. An inverted-U effect of LC-derived norepinephrine may exist, with both low and high levels resulting in reduced exploratory activity. Potentially other stress hormones (e.g. corticosterone) and amygdalar activity, independent of LC innervation, were sufficient to cause the behavioral profile that we observed. Moreover, high levels of exogenous catecholamines can bypass the LC to exert their behavioral effects. Bennett et al. (1990) reported that peripheral injections of epinephrine following DSP-4 treatment can attenuate impairments in active avoidance induced by DSP-4. This indicates that high levels of peripheral catecholamines can bypass the LC noradrenergic system, and our exercise protocol may have been psychologically and physically stressful enough to accomplish this.

The major limitation of this investigation was the exclusion of a no-treadmill control group. We were primarily interested in the influence of forced-exercise, not the influence of the novel treadmill environment. We hypothesized that the novel environment alone may induce plasticity and designed our study to observe the effects of the treadmill exercise beyond the effects of novelty. We did not envision an occlusion of plasticity markers by the novelty; however, not having a home-cage control prevents us from being able to determine if our lack of observed effect on GluR1 phosphorylation and total *Bdnf* was due to the novel

environment masking the effects of acute exercise. Another limitation to this investigation is the absence of a clear understanding of the tissue and extracellular content of norepinephrine in response to exercise and DSP-4. There is enough evidence to support that DSP-4 reduces tissue content of norepinephrine (Ross, 1976; Ögren *et al.*, 1980; Jonsson *et al.*, 1981; Archer *et al.*, 1982; Anisman *et al.*, 1984; Zahniser *et al.*, 1986; Bennett *et al.*, 1990; Scullion *et al.*, 2009; Szot *et al.*, 2010), though the possibility of increased extracellular content of norepinephrine (Ross & Stenfors, 2014) following treatment allows for uncertainty.

Summary: The data presented here indicate that a single acute bout of exercise does not influence GluR1 phosphorylation but does stimulate the transcription of the important plasticity-promoting gene, *Bdnf,* in a transcript-dependent manner. Further, our data suggest that following acute exercise, locomotor and exploratory behavior are reduced and associated with an increase in self-grooming behavior. Importantly, these data suggest that tasks with low intrinsic motivation and dependent on locomotor and exploratory behavior should be avoided when testing for memory or anxiety following acute exercise exposures. Exercise does not increase anxiety or incidents of anxiety attacks in humans and generally appears to be anxiolytic in rodents (O'connor *et al.*, 2000; Sciolino & Holmes, 2012; Ensari *et al.*, 2015), so we are hesitant to conclude that the acute bout of exercise is actually anxiogenic. Instead, it results in behaviors that can be interpreted as an anxious phenotype. Careful consideration should be used

when selecting the appropriate behavioral task following acute exercise exposures.

Chapter 5.

Aim #4: Determine if chronic exercise influences the effect of acute exercise on GluR1 protein phosphorylation and mRNA expression of plasticity-associated genes.

Aim #5: Determine the influence of acute exercise and locus coeruleus noradrenergic signaling on specific *Bdnf* transcript expression.

Title: *Bdnf* Transcription is Differentially Regulated by DSP-4, Acute Forced Treadmill Exercise, and Voluntary Wheel Running

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Introduction

A history of physical activity or exercise training can alter the human and animal response to an acute bout of psychological or physical stress. This modulation of the response to acute physical stress by previous physical activity or exercise training has implications for the application of acute exercise as an effective intervention to improve memory and mental health. Brain-derived neurotrophic factor (BDNF), a neurotrophin critical for exercise-induced brain plasticity (Vaynman *et al.*, 2004), is influenced by previous physical activity and fitness (Knaepen *et al.*, 2010). In humans, acute exercise-induced elevations of peripheral BDNF may be reduced after exercise training (Griffin *et al.*, 2011; Wagner *et al.*, 2015), while research in rodents suggests that short-term exercise training increases hippocampal Bdnf expression to a greater magnitude in mice previously exposed to physical activity (Berchtold *et al.*, 2005). In addition to Bdnf, catecholamines [epinephrine (adrenaline) and norepinephrine (noradrenaline)] are neuromodulators important for mental health and memory performance (McGaugh & Roozendaal, 2002). Acute elevations in catecholamines are associated with improved memory performance and enhanced plasticity (McGaugh, 2013; O'Dell *et al.*, 2015), while loss of normal noradrenergic signaling is associated with age-related memory disorders such as Alzheimer's disease (Szot *et al.*, 2006) Moreover, normal catecholamine signaling is necessary for exercise-induced elevations in Bdnf (Garcia *et al.*, 2003; Ivy *et al.*, 2003). In response to acute exercise of sufficient intensity and duration, catecholamine levels increase to mediate cardiovascular and metabolic

adaptations to physical stress (Tipton, 2006). Importantly, catecholamine responses to acute exercise are influenced by exercise training and fitness (Kjaer, 1998; Zouhal *et al.*, 2008). In trained humans, circulating catecholamines are lower at a given absolute exercise intensity but higher at the same relative or maximal exercise intensity compared to untrained individuals (Dishman & Jackson, 2000; Zouhal *et al.*, 2008). Similarly, rodents exposed to exercise training have a greater capacity for catecholamine release as indicated by increased adrenal weight and adrenaline content (Zouhal *et al.*, 2008), but may have a reduced catecholamine response to acute physical or psychological stress. Indeed, after voluntary wheel running, rodents exposed to acute psychological or physical stress have a lower stress response as determined by reduced stress-induced peripheral and central catecholamine release/depletion (Dishman *et al.*, 1997; 2000; Greenwood *et al.*, 2003) or hypothalamic-pituitary-adrenal (HPA) axis activation (Dishman *et al.*, 1998).

We have previously investigated (Chapter 4) the impact of acute exercise in lifelong sedentary animals on expression of *Bdnf* mRNA and phosphorylation of the GluR1 subunit of the AMPA-type glutamate receptor (AMPAR). The importance of Bdnf expression in hippocampal plasticity is well supported in the literature (for review, see Park & Poo, 2013) and our data demonstrate that a single 30-minute acute bout of high-intensity forced exercise rapidly increases expression of *Bdnf* transcript IV in the mouse hippocampus. Transcription of the *Bdnf* gene is highly complex, producing up to 22 possible transcripts from the nine-exon gene. Of these, *Bdnf* transcript IV appears to be highly sensitive to

physical activity (Gómez-Pinilla *et al.*, 2010; Intlekofer *et al.*, 2013; Venezia *et al.*, 2016).

We hypothesized that acute exercise would increase phosphorylation of Ser845 on the Glur1 subunit of the AMPAR due to an elevation in peripheral catecholamines. Application of exogenous catecholamines increases phosphorylation of Ser845 (chapter 4) and this is associated with a reduced threshold for long-term potentiation (LTP) and enhanced learning (Hu *et al.*, 2007), making it a desirable adaptation. However, in contrast to our previous hypothesis, we did not observe a change in Ser845 phosphorylation in response to acute exercise (chapter 4), potentially due to the short duration of exercise and the short time between the start of exercise and sacrifice of the animal. Importantly, all experiments in the previous investigation were carried out in lifelong sedentary animals. While this design is optimal for understanding the impact of a truly acute bout of exercise, it is not ideal for human application. Exercise should be performed regularly and consistently, and therefore if acute exercise is to be used as a therapeutic or cognitive aid, it is necessary to understand the response to acute exercise in the context of regular voluntary exercise. Our previous data suggest that an acute bout of exercise may be effective at enhancing plasticity and it is essential to understand if these same effects are observed following exercise training.

Noradrenergic signaling plays an essential role in hippocampal Bdnf expression (Garcia *et al.*, 2003; Ivy *et al.*, 2003; Chen *et al.*, 2007; Akhavan *et al.*,

2008). When noradrenergic signaling is blocked, the effect of exercise on *Bdnf* transcription is attenuated (Garcia *et al.*, 2003; Ivy *et al.*, 2003) and remarkably, this extends to *in utero* exercise exposure (Akhavan *et al.*, 2008). Russo-Neustadt et al. (2004) showed that two days of voluntary exercise or reboxetine (selective norepinephrine reuptake inhibitor) both increased total *Bdnf* levels in the rodent hippocampus. Exercise and reboxetine also had unique effects on expression of specific *Bdnf* transcripts. Both treatments alone increased *Bdnf* exon II, and when reboxetine and voluntary exercise were combined there was a robust increase in *Bdnf* transcripts I, II, and IV (referred to as transcript III in the paper) in some or all hippocampal regions examined. These data demonstrate a relationship between exercise, norepinephrine, and *Bdnf* transcription. In the present study, we investigated the influence of one month of voluntary wheel running on the response to an acute bout of forced treadmill exercise. We investigated Ser845 phosphorylation of GluR1, glutamate receptor subunit mRNA expression, and *Bdnf* mRNA expression. Moreover, due to the relationship between norepinephrine and *Bdnf* expression, we investigated the influence of acute exercise and DSP-4-induced noradrenergic lesioning on transcript-specific *Bdnf* expression.

Methods

Mouse model and overview. Male C57BL/6J (Jackson Laboratories, Bar Harbor, ME, USA) mice were used, as they are commonly used to study the impact of exercise on brain phenotypes and in our lab display avid treadmill running activity

and normal physiological responses to exercise training (improved glucose metabolism, lower body mass, increased markers of oxidative capacity, etc.; Ludlow *et al.*, 2012; Guth *et al.*, 2013). All mice were cared for by UMD veterinary staff and kept on 12hr light/12hr dark cycle and provided standard rodent chow. All protocols were approved by the University Institutional Animal Care and Use Committee. We performed two separate experiments to understand how previous physical activity and noradrenergic signaling influence markers of plasticity. In the first experiment, mice were housed with or without access to a freely moving voluntary running wheel for one month before exposure to an acute bout of exercise. In the second experiment, mice were injected with a locus coeruleus-noradrenergic specific neurotoxin (DSP-4) prior to the acute bout of exercise.

Overview of Acute Exercise after One Month of Voluntary Wheel Running Experiment: To determine if one month of voluntary wheel running influences acute exercise-induced markers of hippocampal plasticity, two-month-old C57BL/6J mice were separated into voluntary running and sedentary groups. Voluntary running mice were individually housed in cages containing freely moving running wheels and sedentary mice were individually housed in cages containing locked running wheels. These housing conditions were maintained for one month before acute exercise exposure. Mice were randomly assigned to six groups: 1) sedentary-no acute exercise (CON; n=10); 2) chronically active-no acute exercise (PA-CON; n=9); 3) sedentary-moderate-intensity acute exercise (MOD; n=10); 4) chronically active-moderate-intensity acute exercise (PA-MOD;

n=10); 5) sedentary-high-intensity acute exercise (HI; n=10); 6) chronically active-high-intensity acute exercise (PA-HI; n=10). For three days leading up to the experiment, mice were placed on the stationary treadmill for five minutes per day, during which the electrical stimulus grid at the end of the treadmill belt was activated to familiarize them with the stimulus and treadmill-testing environment. Therefore, they had no previous treadmill running prior to the acute bout, only exposure to the treadmill environment to prevent a response to a novel environment on the experimental day. On day 4, all exercise group mice (MOD, PA-MOD, HI, and PA-HI) were placed on the treadmill, one at a time, and the acute bout of exercise was initiated. Each mouse underwent a six-minute warm up, where the first minute was a no-exercise treadmill exposure; thereafter the treadmill belt began to move at 5 m/min, increasing 1 m/min every minute for five minutes. The treadmill speed was then incrementally increased to the group-appropriate speed and the mouse ran for *45* minutes at this pace. The MOD group ran for 45 minutes at 12 m/min at 0% grade and the HI group ran for 45 minutes at a speed of 18 m/min at 0% grade. Tactile stimulation to the tail was used to encourage mice to run prior to touching the stimulus grid, which reduces the number of stimulus grid touches (unpublished observation). Following the acute bout of exercise, both groups remained on the stationary treadmill for an additional 15 minutes prior to sacrifice. Animals in the CON groups were placed on the stationary treadmill for 66 minutes with the electrical stimulus grid activated.

Overview for DSP-4 Lesioning of Locus Coeruleus and Treadmill Exercise. Three month old male C57Bl/6J mice were separated into four groups: 1) Stationary Treadmill – Saline (CON-SAL; n=9); 2) Stationary Treadmill – DSP-4 (CON-DSP4; n=9); Treadmill Exercise – Saline (EX-SAL; n=7); Treadmill Exercise – DSP-4 (EX-DSP4; n=9). Mice were housed in standard cages with 2-3 mice per cage. Due to fighting, certain mice needed to be separated and individually housed. Individually housed mice are identified in the data figures. Mice underwent the same three-day treadmill familiarization protocol and exercise as described above. Mice ran at a speed between 12 and 18 m/min depending on running ability.

N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4): DSP-4 (Sigma Aldrich) was prepared in 0.9% saline and a single 50 mg/kg dose was delivered by IP injection in a volume of 10 ml/kg; this dose is frequently used in both rats and mice and is effective in depleting hippocampal norepinephrine (Ross & Stenfors, 2014). Control mice received a single IP injection of 0.9% saline. Solutions were prepared for five animals and any remaining solution was discarded. Injections were delivered within 15 minutes of solution preparation and were kept out of the light. Mice received injections seven days prior to treadmill familiarization (10 days prior to experimental treadmill day).

Western Blotting: Twenty-five µg of protein was loaded onto polyacrylamide gels and electrophoresed, followed by transfer to nitrocellulose membranes and immunoblotting. Nitrocellulose membranes were incubated with anti-phospho-

GluR1 (Ser845; Millipore, Billerica, MA) antibody, stripped in a glycine HCl stripping solution and re-probed with anti-Glur1 antibody (Millipore). Because one month of wheel running could potentially increase Glur1 protein expression, we also blotted for Glur1 protein then stripped the membrane and blotted for Gapdh (Millipore). Appropriate fluorescent secondary antibodies were used for detection. A Typhoon scanner (Amersham Biosciences) was used to digitize the fluorescent signal.

Gene Expression: One µg of total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time quantitative PCR (qPCR) was used to assess mRNA expression of *GluR1 (Gria1)*, *NR2B (Grin2b)*, *NR2A (Grin2a)*, *Bdnf* (exon IX), and *Bdnf IV* for the wheel running and acute exercise experiment. qPCR was used to assess mRNA expression of β_2-adrenergic receptor (β_2AR), *Bdnf I, Bdnf II, Bdnf III, Bdnf IV*, *Bdnf VI*, and total *Bdnf* (exon IX) for the DSP-4-lesioning experiment. *Gapdh*, and *ActB* served as expression controls for both experiments. All primer sequences are listed in Appendix A. Primer:probe assays were purchased pre-made *(Gapdh, ActB, NR2A, NR2B, GluR1, β_2AR)* or designed (*Bdnf I*, *Bdnf II*, *Bdnf III*, *Bdnf IV, Bdnf VI,* total *Bdnf*) for the mRNA sequence of each gene using Integrated DNA Technologies' PrimeTime qPCR Assay designer and efficiency tested prior to use. All primer pairs except *Bdnf* total and β_2AR spanned exons to prevent amplification of genomic DNA. Because *Bdnf* total is represented by amplification of only exon IX, this primer pair could not span exons. β_2AR is an

intron-less gene. qPCR data were normalized to the geometric mean of *Gapdh* and *ActB* using the -ΔΔCt method (Vandesompele *et al.*, 2002; Schmittgen & Livak, 2008) and expressed as fold induction ($2^{-\Delta\Delta Ct}$) of mRNA expression compared to the control group (1.0-fold induction).

Statistical Analysis. Protein and mRNA data were analyzed by two-way ANOVA (acute exercise intensity x running wheel or acute exercise x drug treatment) and Tukey's post hoc comparisons when appropriate ($p<0.05$ considered statistically significant).

Results

Acute Exercise after One Month of Voluntary Wheel Running

GluR1. We observed a main effect of the running wheel on GluR1 protein expression ($F_{(1,53)}=5.383$; $p=0.02$) and Ser845 phosphorylation ($F_{(1,53)}=4.287$; $p=0.04$) (Fig. 16). Mice housed with a voluntary running wheel for one month had significantly higher GluR1 protein expression and Ser845 phosphorylation compared to mice housed with a locked wheel. There was no significant effect of running wheel on the ratio of phosphorylated Ser845 over total GluR1 expression. There was no significant effect of acute exercise on GluR1 expression or Ser845 phosphorylation.

Glutamate Receptor mRNA expression: We observed no significant effects of the running wheel or acute exercise on *GluR1, NR2B*, or *NR2A* mRNA expression (Fig. 17).

Total Bdnf mRNA expression. There was a main effect of the running wheel ($F_{(1,52)}$=8.621; p=0.005) and a main effect of acute exercise ($F_{(2,52)}$=3.372; p=0.04) on total *Bdnf* mRNA expression but no running wheel and acute exercise interaction (Fig. 18A). MOD (adjusted p=0.005), HI (adjusted p=0.02), and PA-HI (adjusted p=0.006) had significantly higher *Bdnf* mRNA expression than CON mice. There was no significant difference in total *Bdnf* mRNA expression between mice exposed to moderate and high intensity treadmill running and no differences observed within acute exercise groups (i.e. MOD vs PA-MOD; HI vs PA-HI).

Bdnf IV mRNA expression. There was a main effect of the running wheel ($F_{(1,52)}$=14.59; p=0.0004), a main effect of acute exercise ($F_{(2,52)}$=17.41; p<0.0001), and a running wheel by acute exercise interaction ($F_{(2,52)}$=5.209; p=0.009) for *Bdnf IV* mRNA expression (Fig. 18B). Post hoc analysis revealed that MOD (adjusted p<0.0001) and HI (adjusted p<0.0001) mice had significantly greater *Bdnf IV* mRNA expression compared to CON mice. MOD mice had significantly higher *Bdnf IV* mRNA expression compared to PA-MOD mice (adjusted p=0.002). There was a tendency for PA-HI to have higher *Bdnf IV* expression than CON mice (p=0.07).

Running Performance. Within exercise groups, there was a main effect of acute exercise ($F_{(1,35)}$=19.05; p=0.0001), but no main effect of the running wheel or interaction effect for stimulus pad touches (data not shown). HI intensity runners had significantly more stimulus pad touches than MOD intensity runners but

there were no differences within acute exercise intensity groups (MOD vs PA-MOD or HI vs PA-HI).

DSP-4-Lesioning of Locus Coeruleus and Treadmill Exercise

Total Bdnf (Fig 19A): We observed a main effect of treadmill exercise ($F_{(1,30)}$=6.111; p=0.02) but no effect of the drug treatment or an interaction between treadmill exercise and drug on total *Bdnf* mRNA expression. Post hoc analysis revealed a significant difference between CON-DSP4 and EX-DSP4 (adjusted p=0.03).

We observed no effects of treadmill exercise or drug treatment on *Bdnf I* (Fig 19B), *Bdnf II* (Fig 19C), or *Bdnf III* (Fig 19D) mRNA expression.

Bdnf IV (Fig 19E): We observed a main effect of treadmill exercise ($F_{(1,30)}$=12.92; p=0.001), a main effect of drug treatment ($F_{(1,30)}$=5.106; p=0.03), but no interaction between treadmill exercise and drug treatment on *Bdnf IV* mRNA expression. Post hoc analysis revealed a significant difference between CON-DSP4 and EX-DSP4 (adjusted p=0.02).

Bdnf VI (Fig 19F): We observed a main effect of drug treatment ($F_{(1,30)}$=17.69; p=0.0002) but no effect of treadmill exercise or interaction on *Bdnf VI* mRNA expression. Post hoc analysis revealed a significant difference between EX-SAL and EX-DSP4 (adjusted p=0.006).

We observed no effects of treadmill exercise or drug treatment on β_2*AR* (Fig 20) mRNA expression.

Figure 16. Chronic exercise but not acute exercise increases GluR1 Ser845 phosphorylation and total GluR1 protein expression in the mouse hippocampus. Mice were housed in cages with either locked or freely rotating running wheels for one month and exposed to 45 minutes (after 6-minute warm up) of moderate-intensity (12m/min; n=20; 10 runners 10 sedentary), high-intensity (18m/min; n=20; 10 runners 10 sedentary), or no exercise treadmill exposure (n=20; 10 runners 10 sedentary) followed by 15 minutes of rest on the treadmill. Mice were sacrificed and hippocampi isolated immediately after the 15-minute rest. Mice housed with voluntary running wheels had significantly more Ser845 phosphorylation compared to sedentary mice ($F_{(1,53)}$=4.287; p=0.04). Mice housed with voluntary running wheels also had significantly more GluR1 expression compared to sedentary mice ($F_{(1,53)}$=5.383; p=0.02). There was no effect of voluntary wheel running on the ratio of pSer845 over GluR1. There was no effect of acute exercise on GluR1 expression or phosphorylation. Error bars represent SEM. * denotes significance at p<0.05.

Figure 17. Acute and chronic exercise do not influence glutamate receptor subunit mRNA expression. Mice were housed in cages with either locked or freely rotating running wheels for one month and exposed to 45 minutes (after 6-minute warm up) of moderate-intensity (12m/min; n=20; 10 runners 10 sedentary), high-intensity (18m/min; n=20; 10 runners 10 sedentary), or no exercise treadmill exposure (n=20; 10 runners 10 sedentary) followed by 15 minutes of rest on the treadmill. Mice were sacrificed and hippocampi isolated immediately after the 15 minute rest. Target mRNA expression is presented as

$2^{-\Delta\Delta Ct}$ relative to the geometric mean of *ActB* and *Gapdh*. There was no effect of acute exercise or running wheel on *GluR1* (A), *NR2B* (B), or *NR2A* (C).

Figure 18. Acute and chronic exercise increase total *Bdnf* and *Bdnf IV* mRNA expression Mice were housed in cages with either locked or freely rotating running wheels for one month and exposed to 45 minutes (after 6-minute warm up) of moderate-intensity (12m/min; n=20; 10 runners 10 sedentary), high-intensity (18m/min; n=20; 10 runners 10 sedentary), or no exercise treadmill exposure (n=20; 10 runners 10 sedentary) followed by 15 minutes of rest on the treadmill. Target mRNA expression is presented as $2^{-\Delta\Delta Ct}$ relative to the geometric mean of *ActB* and *Gapdh*. A) Total *Bdnf*: There was a main effect of running wheel ($F_{(1,52)}$=8.621; p=0.005) and a main effect of acute exercise ($F_{(2,52)}$=3.372; p=0.04) but no wheel running x acute exercise interaction. B) *Bdnf IV*. There was a main effect of running wheel ($F_{(1,52)}$=14.59; p=0.0004), a main effect of acute exercise ($F_{(2,52)}$=17.41; p<0.0001), and a wheel running x acute exercise interaction ($F_{(2,52)}$=5.209; p=0.009). Error bars represent SEM. * denotes significantly different than CON (p<0.05). $ denotes significant difference from CON housing within acute exercise groups (p<0.05).

Figure 19. Acute exercise and DSP-4 lesioning influence Bdnf transcript expression. Mice were injected with either saline or DSP-4 ten days prior to exposure to 45 minutes (after 6-minute warm up) of treadmill exercise (n=16; 7 saline 9 DSP-4) or no exercise treadmill exposure (n=18; 9 saline 9 DSP-4) followed by 15 minutes of rest on the treadmill. Target mRNA expression is

presented as $2^{-\Delta\Delta Ct}$ relative to the geometric mean of *ActB* and *Gapdh*. A) Total *Bdnf*: There was a main effect of treadmill exercise ($F_{(1,30)}$=6.111; p=0.02) but no effect of the drug treatment or treadmill x drug interaction. B) *Bdnf I*: There was no influence of exercise or drug on Bdnf *I* mRNA expression. C) *Bdnf II*: There was no influence of exercise or drug on Bdnf II mRNA expression. D) *Bdnf III*: There was no influence of exercise or drug on *Bdnf III* mRNA expression. E) *Bdnf IV*: There was a main effect of treadmill exercise ($F_{(1,30)}$=12.92; p=0.001), a main effect of drug treatment ($F_{(1,30)}$=5.106; p=0.03), but no treadmill x drug interaction. F) *Bdnf VI*: There was a main effect of drug treatment ($F_{(1,30)}$=17.69; p=0.0002) but no effect of treadmill exercise or treadmill x drug interaction. * indicates significantly different (p<0.05). x's represent data points of animals that were individually housed due to fighting with cage mates.

Figure 20. β_2-adrenergic receptor mRNA expression is not influenced by DSP-4 treatment or acute exercise. Mice were injected with either saline or DSP-4 ten days prior to exposure to 45 minutes (after 6 minute warm up) of treadmill exercise (n=16; 7 saline 9 DSP-4) or no exercise treadmill exposure (n=18; 9 saline 9 DSP-4) followed by 15 minutes of rest on the treadmill. Target mRNA expression is presented as $2^{-\Delta\Delta Ct}$ relative to the geometric mean of *ActB* and *Gapdh*. There was no effect of acute exercise or DSP-4 on β_2*AR* mRNA expression

Figure 16.

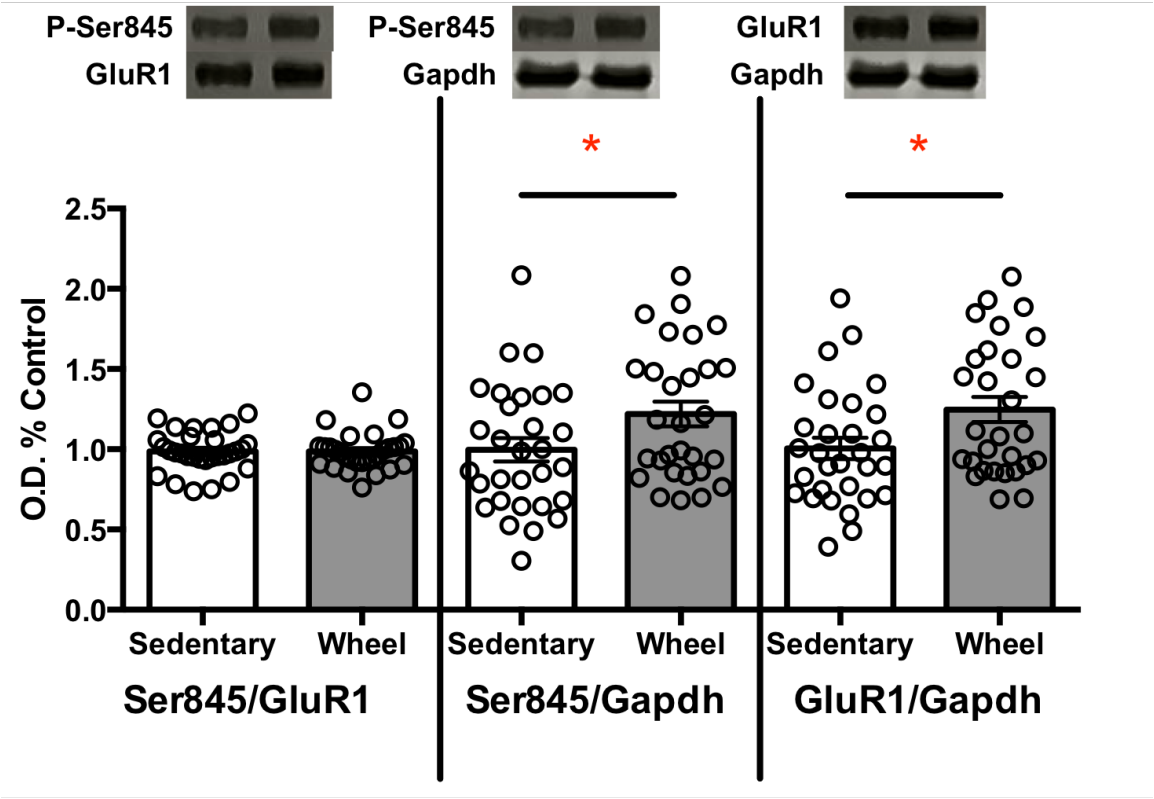

P-Ser845
GluR1
P-Ser845
Gapdh
GluR1
Gapdh
*
*
O.D. % Control
2.5
2.0
1.5
1.0
0.5
0.0
Sedentary
Wheel
Sedentary
Wheel
Sedentary
Wheel
Ser845/GluR1
Ser845/Gapdh
GluR1/Gapdh

Figure 17.

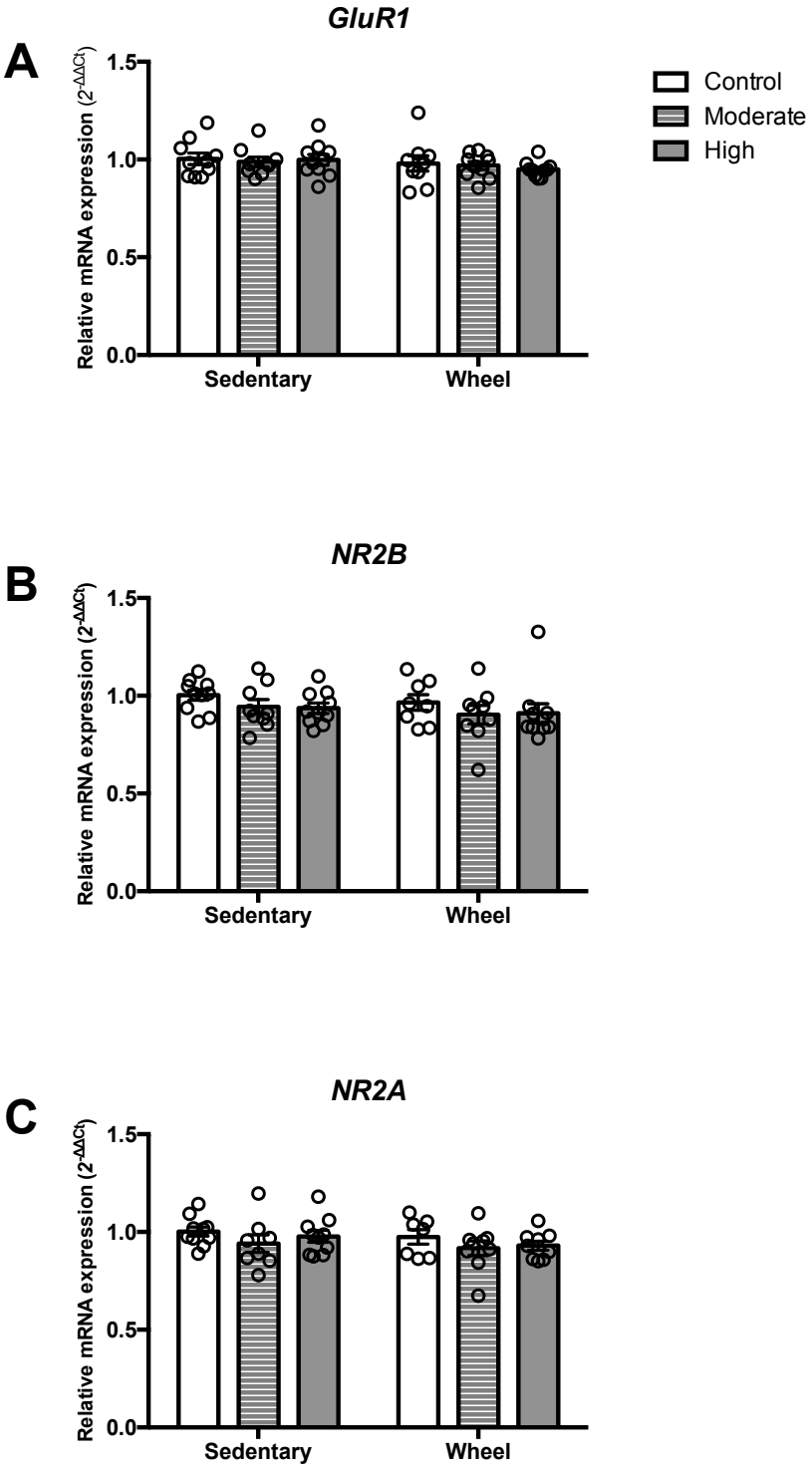

A
GluR1
Relative mRNA expression ($2^{-\Delta\Delta Ct}$)
1.5
1.0
0.5
0.0
Sedentary
Wheel
Control
Moderate
High
B
NR2B
Relative mRNA expression ($2^{-\Delta\Delta Ct}$)
1.5
1.0
0.5
0.0
Sedentary
Wheel
C
NR2A
Relative mRNA expression ($2^{-\Delta\Delta Ct}$)
1.5
1.0
0.5
0.0
Sedentary
Wheel

Figure 18

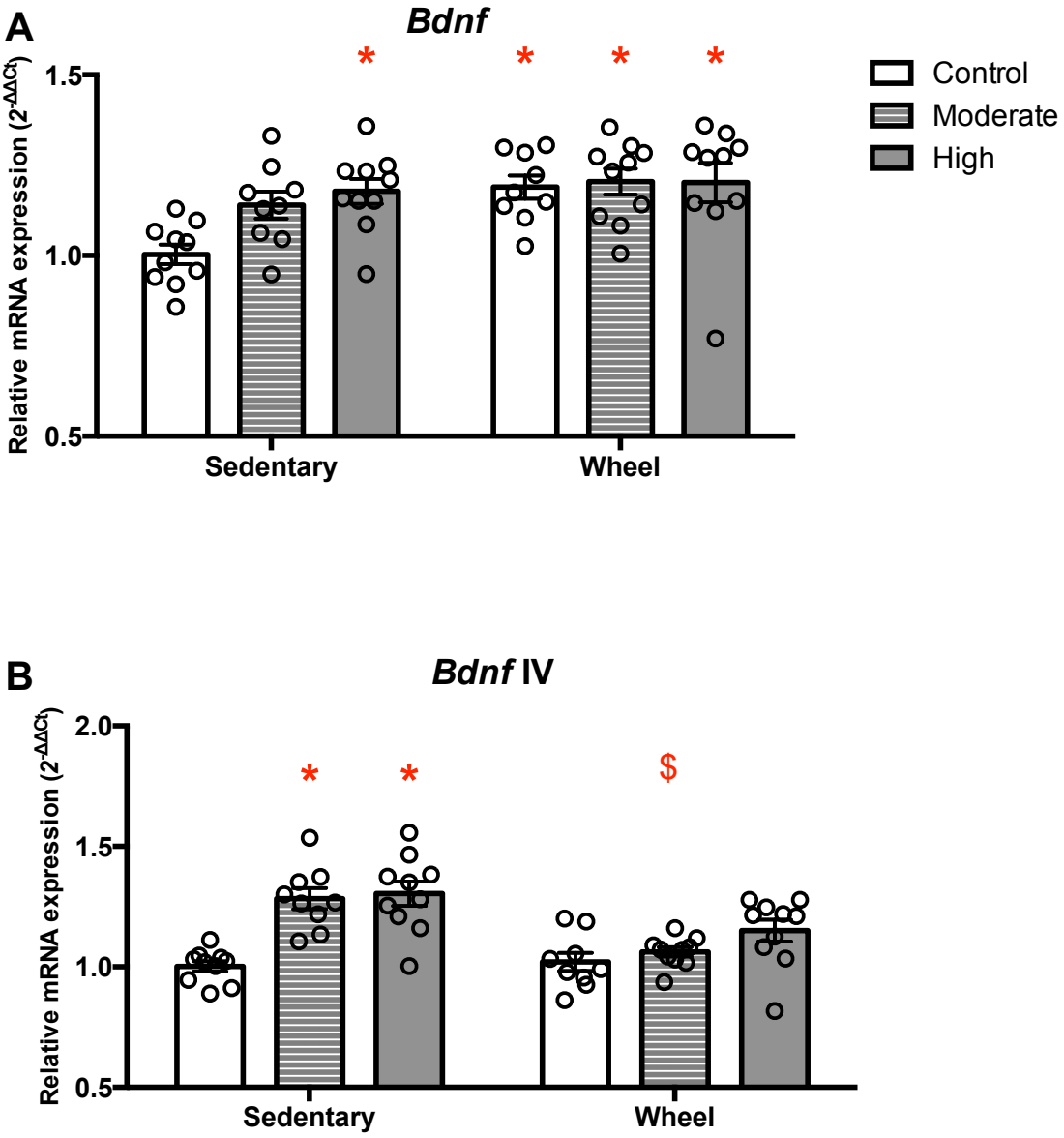
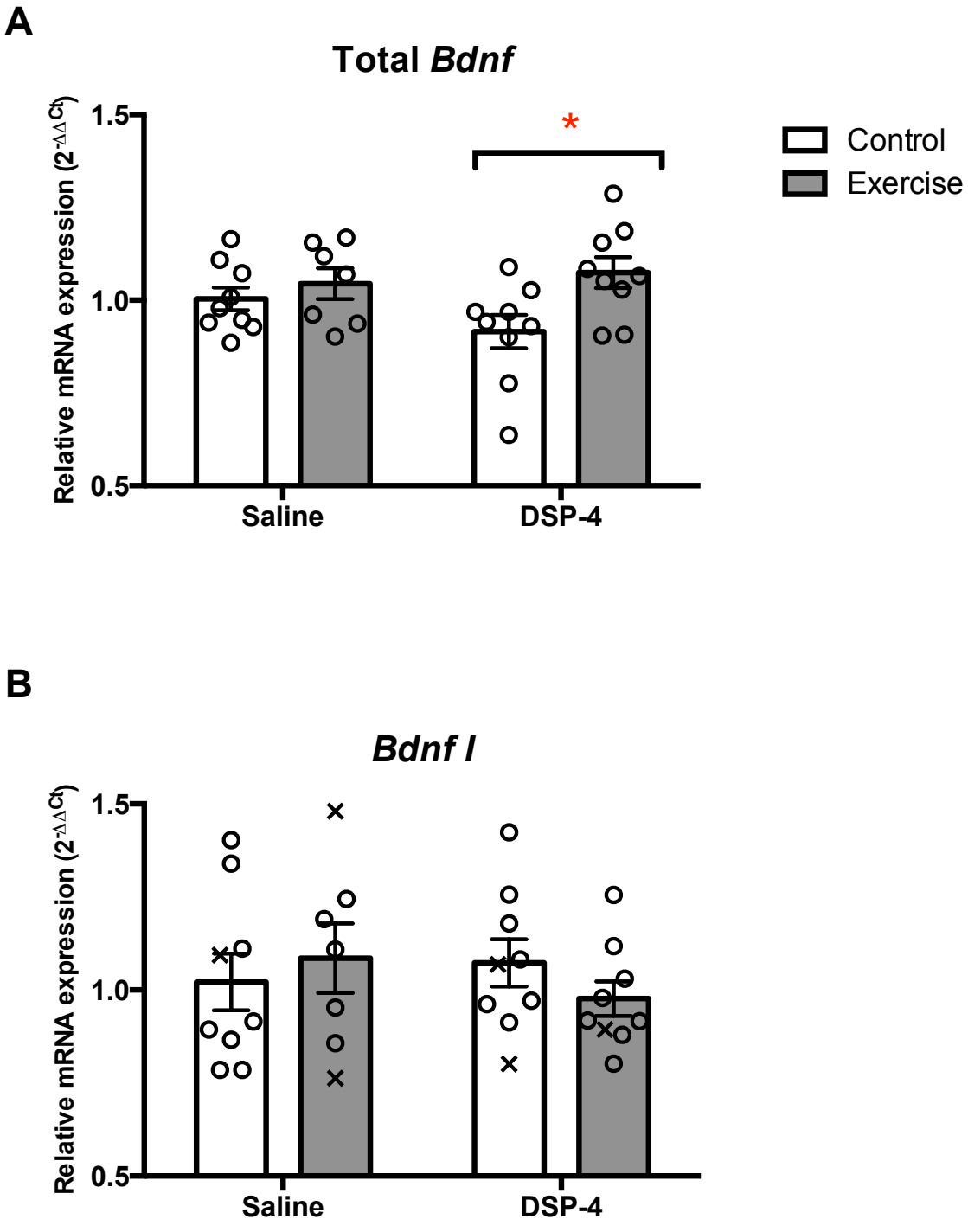
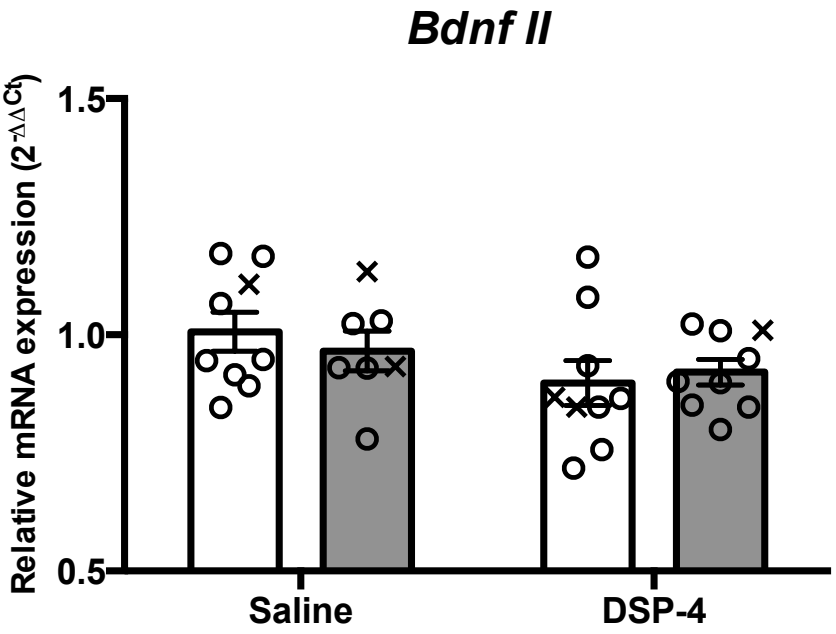

A
Bdnf
Relative mRNA expression (2-ΔΔCt)
1.5
1.0
0.5
*
*
*
*
Sedentary
Wheel
Control
Moderate
High
B
Bdnf IV
Relative mRNA expression (2-ΔΔCt)
2.0
1.5
1.0
0.5
*
*
$
Sedentary
Wheel

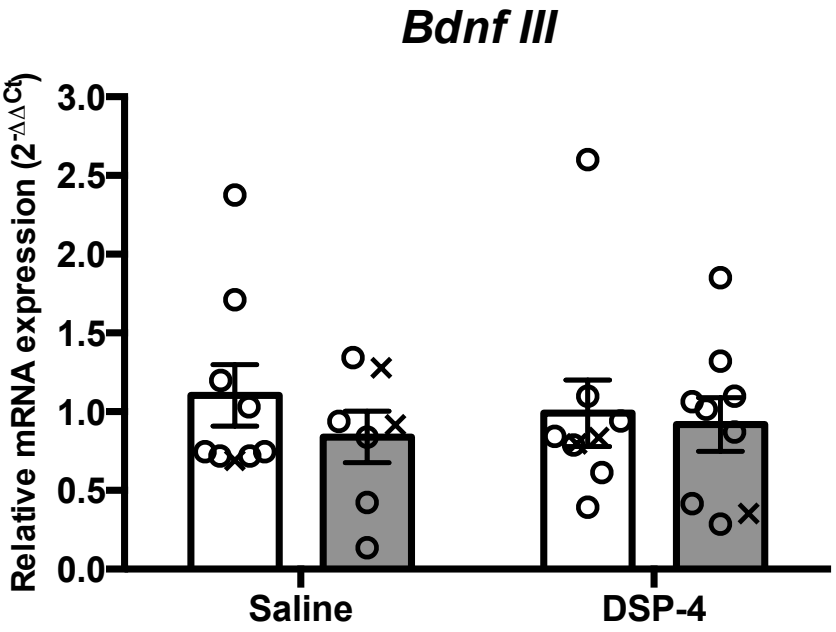
Figure 19


A
Total *Bdnf*
Relative mRNA expression ($2^{-\Delta\Delta Ct}$)
1.5
1.0
0.5
*
Saline
DSP-4
Control
Exercise
B
Bdnf I
Relative mRNA expression ($2^{-\Delta\Delta Ct}$)
1.5
1.0
0.5
Saline
DSP-4

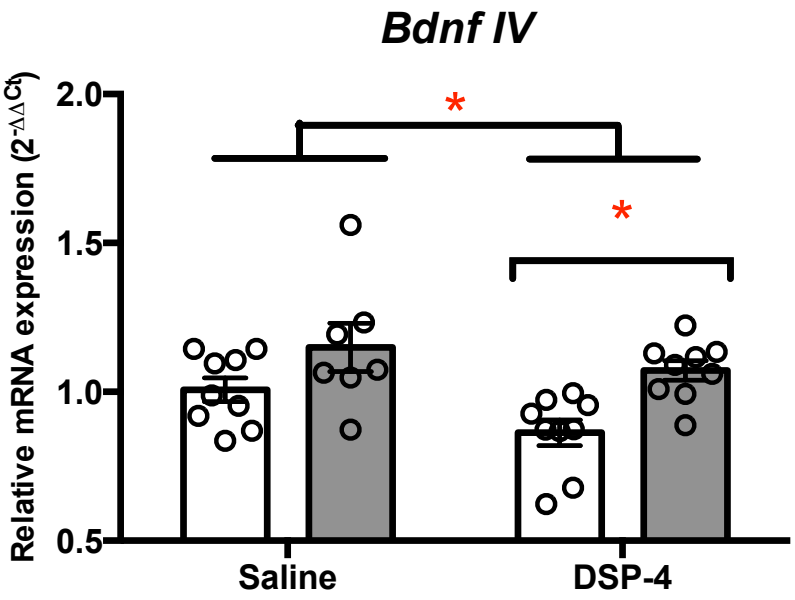
C


Bdnf II
Relative mRNA expression (2-ΔΔCt)
1.5
1.0
0.5
Saline
DSP-4

D


Bdnf III
Relative mRNA expression (2-ΔΔCt)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Saline
DSP-4

E


Bdnf IV
Relative mRNA expression (2-ΔΔCt)
2.0
1.5
1.0
0.5
*
*
Saline
DSP-4

F

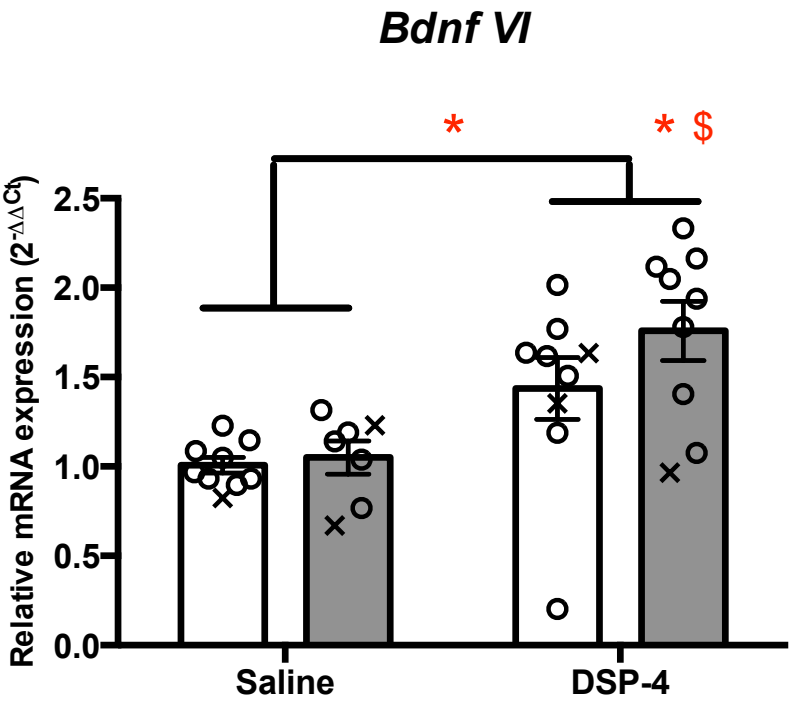
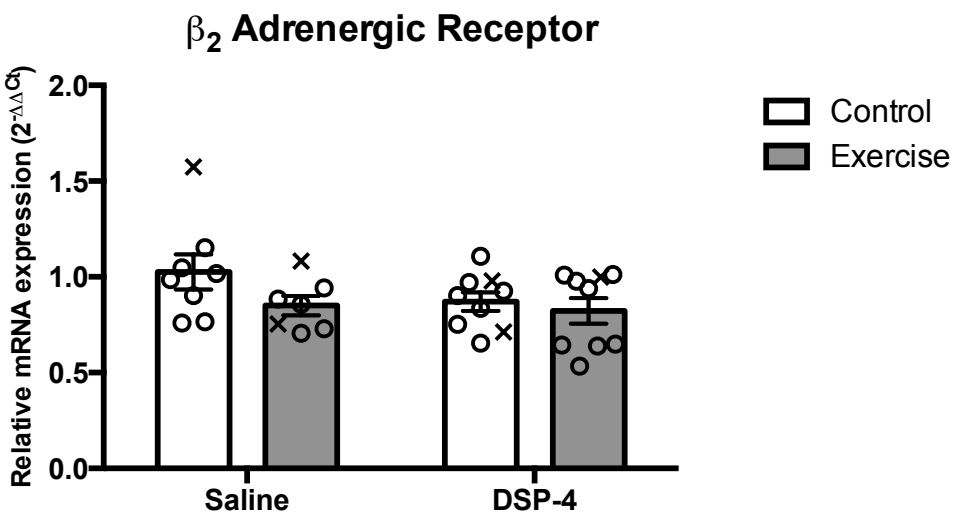

Bdnf VI
Relative mRNA expression (2-ΔΔCt)
2.5
2.0
1.5
1.0
0.5
0.0
*
* $
Saline
DSP-4

Figure 20


β2 Adrenergic Receptor
Relative mRNA expression (2-ΔΔCt)
2.0
1.5
1.0
0.5
0.0
Saline
DSP-4
Control
Exercise

Discussion

We observed that one month of voluntary wheel running increased expression of GluR1 protein and phosphorylation at Ser845. Moreover, we confirmed our previous findings (Chapter 4) that acute forced treadmill exercise increased *Bdnf IV* mRNA expression, though remarkably, this effect of acute exercise was attenuated by one-month of voluntary wheel running. We also observed that both one month of chronic voluntary wheel running and 45 minutes of acute exercise at either high or moderate intensity increased expression of total *Bdnf* mRNA. We hypothesized that the blunting of *Bdnf IV* expression after one month of voluntary wheel running was due to reduced noradrenergic activity after exercise, so we lesioned the locus coeruleus-noradrenergic system with DSP-4 prior to the acute exercise. Contrary to our hypothesis, acute exercise increased *Bdnf IV* and total *Bdnf* in DSP-4 treated mice. Additionally, we found that DSP-4 increased expression of *Bdnf* transcript VI.

Glutamate Receptors: We observed a significant increase in phospho-Ser845 following one month of voluntary wheel running. An increase in phosphorylated Ser845 is indicative of more membrane-inserted AMPA receptors and is believed to reduce the threshold for synaptic plasticity and learning (Hu *et al.*, 2007; Makino *et al.*, 2011). We also observed a significant increase in total GluR1 protein expression. Mizutani et al. (2015) found higher levels of Ser845 phosphorylation in a model of cortical infarction following one week of voluntary wheel running but observed no effect on total GluR1 protein expression.

Differences in the brain region, length of wheel running exposure, and health of the tissue may account for the differences between our investigation and Mizutani et al. (2015). Indeed, Dietrich et al. (2005) reported that four weeks of voluntary wheel running (the same duration of wheel exposure as in the current investigation) increased protein levels of GluR1 in the mouse cortical post synaptic density, suggesting high levels of Ser845 phosphorylation. In the current investigation, the increase in GluR1 protein was of similar magnitude with the elevation in Ser845 phosphorylation and therefore we did not observe any differences in the ratio of phospho-Ser845 over total GluR1. Upon exogenous catecholamine delivery or psychological stress there is an increase in the ratio of Ser845 to total GluR1 [Chapter 4, (Hu *et al.*, 2007)]. We believe our observed response to be a favorable adaptation to chronic exercise because maintaining a low ratio of phospho-Ser845 to GluR1, while increasing both phospho-Ser845 and GluR1 protein may result in more membrane-inserted AMPARs and also more available GluR1 containing AMPARs to be phosphorylated and trafficked to the synapse in response to an appropriate stimulus.

We did not observe a significant effect of acute exercise on Ser845 phosphorylation, which is consistent with our previous investigation (Chapter 4) that examined mice sacrificed immediately after exposure to 30 minutes of acute exercise. Potentially, the acute bout of exercise was not sufficient in intensity or duration to lead to a widespread elevation of norepinephrine [or dopamine, which also has the potential to phosphorylate Ser845 (Price *et al.*, 1999)] throughout the hippocampus. Moreover, we used whole hippocampal homogenates.

Potentially, acute forced treadmill running influences the hippocampus in a region-specific manner. Numerous studies suggest that the dorsal hippocampus is important for spatial memory and the ventral hippocampus is important for anxiety-like behaviors (Bannerman *et al.*, 2004) and running has been shown to affect the rodent ventral and dorsal hippocampus differently (Schoenfeld *et al.*, 2013). Therefore, it is possible that the effects of our acute bout of forced exercise may differ along the longitudinal axis of the hippocampus.

The increase in GluR1 protein with one month of voluntary wheel running was not consistent with our qPCR findings of GluR1 mRNA, which was not different from sedentary controls. We also did not observe an effect of wheel running or acute exercise on NMDA receptor subunits. Molteni et al. (2002) reported that three days of voluntary wheel running increased NR2B and NR2A mRNA expression in the rat hippocampus, though these were no longer different than controls after 28 days of wheel running. Potentially, we would have observed an effect on glutamate receptor subunit expression at an earlier time point. The observation that neither chronic exercise nor 45 minutes of acute exercise increased expression of glutamate receptor subunit mRNA expression is consistent with our previous investigations (Chapters 3 and 4; Venezia *et al.*, 2016).

Bdnf transcription: We observed a significant effect of voluntary wheel running and acute exercise on total *Bdnf* expression. Both one month of voluntary wheel running and acute treadmill running increased expression of total *Bdnf* mRNA

with no interaction between the exercise stimuli. It is well supported in the literature that one month of voluntary wheel running increases hippocampal *Bdnf* mRNA (for review, see Cotman *et al.*, 2007; Voss *et al.*, 2013), however the influence of one bout of acute exercise has not been thoroughly investigated. In contrast to the results observed immediately following 30 minutes of acute exercise in our previous investigation (Chapter 4), 45 minutes of running followed by 15 minutes of rest resulted in an intensity-independent increase in total *Bdnf* mRNA. This suggests that 30 minutes of acute exercise was not sufficient in duration and that either 45 minutes of exercise or a 60-minute delay from the initiation of exercise to sacrifice is required. Interestingly, both acute and chronic exercise increased *Bdnf* transcription to similar levels with no additive effect of the two. This indicates that one bout of acute exercise is equally effective at increasing this important neurotrophin as one month of voluntary wheel running. This finding requires further investigation to determine if the effects on total *Bdnf* mRNA expression are more persistent following chronic exercise than following an acute bout of exercise. Berchtold et al. (2005) reported that even though both daily and intermittent (every other day) exposure to voluntary wheel running increased Bdnf protein expression to similar levels, daily wheel running resulted in more persistent elevation in Bdnf levels.

Both acute and chronic exercise significantly influenced *Bdnf IV* expression; however, in contrast to total *Bdnf*, there was an interaction between voluntary wheel running and acute exercise. Moderate- and high-intensity acute exercise increased *Bdnf IV* mRNA only in mice housed with a locked running

wheel. A history of physical activity has previously been shown to influence Bdnf protein expression in response to short-term exercise exposure. Berchtold et al. (2005) showed that two days of wheel running increased Bdnf protein expression only in mice that were previously exposed to 14 days of wheel running. Further, Gomez-Pinilla et al. (2010) reported that one week of voluntary wheel running decreased *Bdnf IV* promoter methylation. A reduction in promoter methylation suggests greater transcriptional capacity following a short exposure to activity. These two studies suggest that a previous history of physical activity increases the capacity to induce *Bdnf* transcription; however, we saw a blunting of *Bdnf IV* expression after one month of voluntary wheel running. This suggests that a signaling mechanism upstream of *Bdnf IV* transcription is downregulated following voluntary wheel running. We hypothesized that the modulated signaling mechanism is exercise-induced noradrenergic signaling.

Noradrenergic signaling is an important regulator of Bdnf expression. Application of norepinephrine to neurons in culture or injection of exogenous norepinephrine *in vivo* can increase expression of Bdnf (Chen *et al.*, 2007; Mello-Carpes *et al.*, 2016), similar to what is observed following treatment with antidepressants, including norepinephrine reuptake inhibitors such as reboxetine (Russo-Neustadt *et al.*, 2004; Larsen *et al.*, 2008; Musazzi *et al.*, 2009; Baj *et al.*, 2012). Exercise-induced *Bdnf* transcription is dependent on β-adrenergic receptor (Ivy *et al.*, 2003) and normal LC-noradrenergic signaling (Garcia *et al.*, 2003). If either of these are compromised, exercise no longer increases *Bdnf* expression (Garcia *et al.*, 2003; Ivy *et al.*, 2003). Further, though exercise

training is associated with an increased capacity for catecholamine release (Zouhal *et al.*, 2008), a previous history of physical activity is associated with a reduced stress response (Dishman *et al.*, 1997; 1998; 2000; Greenwood *et al.*, 2003) For example, norepinephrine release/depletion in the LC and hippocampus in response to treadmill or immobilization stress is reduced following six weeks of treadmill training (Dishman *et al.*, 2000). This led us to hypothesize that the blunting of *Bdnf IV* expression following one month of voluntary wheel running was due to reduced acute exercise-induced noradrenergic signaling after wheel exposure. Our analysis revealed that acute exercise increased total *Bdnf* and *Bdnf IV* only in DSP-4 treated mice (though there was a main effect of exercise). This is interesting since Garcia et al. (2003) reported that DSP-4 prevented the voluntary wheel running-induced increase in total *Bdnf* expression but did not decrease *Bdnf* expression in sedentary controls. This is in agreement with the effects of propranolol, a βAR-antagonist, on exercise induced *Bdnf* expression (Ivy *et al.*, 2003). We speculate that the treadmill environment alone induced *Bdnf* and *Bdnf IV* expression through noradrenergic signaling and DSP-4 attenuated this; however, acute high-intensity exercise was sufficient to overcome the reduction in LC-derived norepinephrine, potentially through LC-norepinephrine independent pathways such as strong synaptic activity at glutamatergic synapses or serotonergic signaling. Indeed, DSP-4 is reported to leave serotonergic signaling intact, and serotonin signaling may also be important for exercise-induced *Bdnf* expression (Ivy *et al.*, 2003; Russo-Neustadt *et al.*, 2004).

Interestingly, in the Garcia et al. (2003) investigation, one week of voluntary exercise did not increase *Bdnf IV* but DSP-4 and DSP-4 plus one week of exercise did increase *Bdnf IV*. Again, this increase with DSP-4 alone and DSP-4 plus exercise relative to saline controls is inconsistent with our findings and might be explained by our mice being exposed to the treadmill immediately before sacrifice and differences between one-week of voluntary wheel running and an acute bout of forced exercise. We also observed an increase in *Bdnf VI* expression with DSP-4 treatment. Interestingly, Russo-Neustadt et al. (2004) reported that *Bdnf VI* was reduced following one week of reboxetine or combined reboxetine and exercise. Reboxetine is a norepinephrine reuptake inhibitor and should increase norepinephrine availability, especially when combined with exercise, whereas DSP-4 should reduce norepinephrine availability. Therefore, it appears that high levels of norepinephrine reduce *Bdnf VI* expression while low levels increase *Bdnf VI* expression. Understanding why DSP-4 plus acute exercise in the current investigation increased *Bdnf VI* more than exercise alone requires further investigation.

We did not observe an effect of acute exercise or DSP-4 on *Bdnf I*, *II*, or *III*. This is interesting since *Bdnf I* has been shown to be influenced by exercise (Oliff *et al.*, 1998; Garcia *et al.*, 2003; Russo-Neustadt *et al.*, 2004; Intlekofer *et al.*, 2013), while Bdnf II expression is increased by norepinephrine, norepinephrine reuptake inhibitors, and exercise (Russo-Neustadt *et al.*, 2004; Zajac *et al.*, 2009; Musazzi *et al.*, 2014). Garcia et al. (2003) reported that one week of voluntary wheel running increased expression of *Bdnf I,* which was attenuated with DSP-4,

and *Bdnf II*, which was potentiated with DSP-4. We did not observe an effect of either DSP-4 or acute exercise on *Bdnf I* or *Bdnf II*. *Bdnf I* is sensitive to exposure to novel contexts (Lubin *et al.*, 2008), so even though *Bdnf I* is sensitive to acute exercise (Oliff *et al.*, 1998) and stress (Marmigère *et al.*, 2003), the treadmill environment alone may have been sufficient to increase expression and mask the effects of exercise. It is possible that multiple days of exercise (Zajac *et al.*, 2009) or very long acute exercise protocols (Oliff *et al.*, 1998) are necessary to increase expression of Bdnf II and III.

Summary: Our data support that one truly acute bout of exercise increases expression of total *Bdnf* and *Bdnf* transcript IV; however, previous exposure to voluntary wheel running may reduce the effectiveness of acute exercise to increase *Bdnf IV* expression. Our data further demonstrate that acute exercise has the capacity to increase *Bdnf* transcription even with compromised noradrenergic signaling, which has implications for the treatment of disorders associated with reduced noradrenergic signaling and LC neurodegeneration such as Alzheimer's disease (Szot *et al.*, 2006). We also report that increased Ser845 phosphorylation and expression of GluR1 protein may be a potential mechanism by which exercise training increases hippocampal plasticity, though these adaptations are not induced by a single acute bout of exercise.

Chapter 6. Summary, Conclusions, Limitations, and Future Directions

Overall Summary: The overall aim of this dissertation research was to explore the mechanisms by which exercise influences brain health, specifically focusing on the hippocampus, a brain region critical for memory and emotional health. The dissertation reports the findings from three investigations, each exploring the influence of physical activity on markers of hippocampal plasticity, through three unique approaches. The dissertation provides a comprehensive look at the influence of different exercise exposures, from a single exposure of acute forced exercise to long-term voluntary exercise. The first investigation explored how long-term physical activity influences markers of hippocampal plasticity, which has implications for understanding why people who are physically active throughout their lifetime maintain brain health into old age. In addition to being uniquely long in comparison to the majority of existing published research (i.e. 20 weeks vs. a typical range of seven to 28 days), this investigation was also unique because it included both male and female mice. We observed sex differences in the influence of long-term exercise exposure, with exercise-induced increases in Bdnf protein and mRNA expression only detected in male mice (Chapter 3, Figs. 3 & 6). Further research is required to uncover the mechanisms associated with the observed sex differences. We speculate that sex differences in exercise-induced Bdnf expression were due to sex hormones or differences observed in running behavior, since males ran significantly more during the last week of wheel exposure and maintained their running levels throughout the exposure more than females (Chapter 3, Fig. 1). This is

noteworthy since most studies use shorter chronic exercise protocols and capitalize on the high wheel activity that is observed during the first few weeks of wheel exposure. Treadmill running might be a more appropriate approach for investigating how long-term exercise influences brain health in rodents since the volume and intensity of exercise can be manipulated and maintained.

Though we observed sex differences in total Bdnf mRNA and protein expression in response to five-months of voluntary wheel running, *Bdnf* transcript IV expression was increased in both sexes (Chapter 3, Fig. 3). *Bdnf IV* mRNA content increased following 30 minutes of high-intensity (Chapter 4, Fig. 10) or 45 minutes of high- or moderate-intensity forced treadmill running (Chapter 5, Figs 18&19) and was supported in three separate experiments, providing strong evidence that acute exercise promotes plasticity by increasing expression of this rapidly transcribed *Bdnf* transcript. Acute exercise also increased total *Bdnf* expression when performed for 45 minutes but not for 30 minutes (Chapters 4 & 5, Figs. 10, 18, & 19). Interestingly, while five months of voluntary wheel running and one bout of acute forced exercise increased *Bdnf IV* expression, one month of voluntary wheel running was not sufficient to increase expression of *Bdnf IV* and actually prevented the acute exercise-induced transcription (Chapter 5, Fig. 18). The blunting effect of one month of voluntary wheel running was not observed in total *Bdnf* (Chapter 5, Fig. 18). Lesioning the locus coeruleus with DSP-4 also reduced *Bdnf IV* expression, but remarkably, this was rescued by an acute bout of forced exercise (Chapter 5, Fig. 19) The rescue of *Bdnf IV* expression with acute exercise has important implications for exercise in the

aged population and individuals suffering from Alzheimer's Disease who experience neurodegeneration of the locus coeruleus (Chalermpalanupap *et al.*, 2013). These results demonstrate that exercise can increase *Bdnf* expression even when challenged by a compromised noradrenergic system.

Though acute exercise increases expression of *Bdnf*, a neurotrophin that is necessary for hippocampal plasticity and memory, we were unable to determine if this increase is associated with improved memory. Mice exposed to a memory task immediately after acute forced treadmill running failed to perform the task due to significantly reduced exploratory behavior (Chapter 4, Fig. 12). We tested whether this reduction in exploratory behavior would also be observed in a traditional anxiety task that utilizes a similar testing environment as our memory task. Once again, acute forced treadmill running reduced exploratory behavior (Chapter 4, Figures 13, 14, & 15). Interestingly, lesioning the noradrenergic system partially attenuated this effect. Mice injected with DSP-4 and exercised did not show reduced exploratory behavior compared to mice injected with DSP-4 and exposed to the stationary treadmill. However, they did have significantly less activity than saline injected controls and therefore it is not possible to say that lesioning the LC rescued this behavioral phenotype. Interestingly, exercised mice also spent significantly more time self-grooming and this was not attenuated by DSP-4 (Chapter 4, Figures 13, 14, & 15). The finding that acute exercise increases anxious behavior in mice and prevents them from performing exploratory-based memory tasks highlights the obstacles faced when trying to determine the effectiveness of acute exercise at improving memory or

reducing anxiety. Non-locomotor dependent tasks that do not rely heavily on exploratory behavior (e.g. virtual reality) and/or contain a high level of intrinsic motivation should be strongly considered when selecting behavioral tasks to be performed after exercise exposures. A potential option for a memory task is a one-trial spatial learning task that relies on sexual drive of male mice to identify the location of previously present female mice (Meier *et al.*, 2010; Fellini & Morellini, 2013)

Another consistent finding in all three studies is that exercise has limited or no effects on hippocampal mRNA expression of glutamate receptor subunits. The data indicate no changes in *GluR1*, *NR2A*, or *NR2B* mRNA expression after one (Chapter 5, Fig. 17) or five (Chapter 1, Fig. 4) months of voluntary wheel running, or 30 (Chapter 4, Fig. 9) or 45 (Chapter 5, Fig. 17) minutes of acute forced exercise. Potentially, these mRNAs are expressed at such high levels in the hippocampus that changes with exercise are difficult to detect, especially in whole hippocampal homogenates. One month of voluntary wheel running did increase the expression of GluR1 protein and its phosphorylation at Ser845 (Chapter 5, Fig. 16), which is a favorable adaptation and should be investigated further. For example, by keeping the ratio of phosphorylated Ser845 to total GluR1 low but increasing absolute values of each, potentially the synapse has increased synaptically inserted AMPARs, yet in response to the appropriate stimulus (e.g. IP injection of epinephrine), rapid phosphorylation of available GluR1 at Ser845 can increase peri-synaptic insertion of GluR1-containing AMPARs. In contrast to our hypothesis, one bout of acute exercise did not

induce phosphorylation of Ser845 and this was confirmed by two experiments (Chapters 4 & 5).

Overall, this dissertation provides strong and convincing evidence that both acute and chronic exercise increase expression of *Bdnf* in the mouse hippocampus. Though the literature supports that short-term (seven to 28 days) exercise increases *Bdnf* transcription in the hippocampus, the effectiveness of one bout of exercise and/or long-term wheel running (>90 days) in adult rodents was previously unknown. This dissertation also provides evidence that exercise has little or no effect on glutamate receptor expression or phosphorylation of GluR1, though the effects may be subtle and difficult to detect with the methodological approaches utilized in these investigations. It also provides "food for thought" concerning the selection and timing of behavioral tasks when exploring behavioral adaptations to exercise.

Limitations and Considerations: We did not have the capability to comprehensively examine the influence of DSP-4 treatment in our experiments. There are some inconsistencies reported in the literature on the influence of DSP-4 on both behavior and extracellular norepinephrine. Though DSP-4 dramatically reduces tissue levels of norepinephrine in regions innervated by the LC such as the hippocampus (Ross, 1976; Ögren *et al.*, 1980; Jonsson *et al.*, 1981; Archer *et al.*, 1982; Anisman *et al.*, 1984; Zahniser *et al.*, 1986; Bennett *et al.*, 1990; Scullion *et al.*, 2009; Szot *et al.*, 2010), increased extracellular norepinephrine in the frontal cortex (Kask *et al.*, 1997; Hughes & Stanford,

1998a; 1998b) and elevated β-adrenergic receptor expression in the hippocampus (Zahniser *et al.*, 1986) have also been reported. The increased extracellular norepinephrine in brain regions innervated by the LC, despite dramatic reductions in dopamine beta hydroxylase (rate-limiting enzyme in norepinephrine synthesis) and tissue content of norepinephrine, are potentially due to non-LC noradrenergic neurons releasing norepinephrine into the extracellular space and the inability of LC terminals to take up the norepinephrine in the surrounding region (Ross & Stenfors, 2014). DSP-4 exerts its effect by irreversibly disrupting the norepinephrine transporter (Ross & Stenfors, 2014). In contrast to reports of elevated extracellular norepinephrine after DSP-4 treatment, others have shown that corticotropin-releasing factor (CRF) -stimulated norepinephrine release from the LC is greatly reduced in rats pre-treated with DSP-4 (Zhang *et al.*, 1998; Palamarchouk *et al.*, 2000). Together, the research on DSP-4 suggests that there may be an elevation in basal extracellular norepinephrine, though stress induced norepinephrine release in the hippocampus is attenuated with pre-treatment of DSP-4. There was no change in β-adrenergic receptor expression in our sample but we did not have the capability to comprehensively explore tissue content and extracellular levels of norepinephrine in these investigations. Previous research on DSP-4, which spans decades, combined with our data suggest that the drug was effective at lesioning the LC in our animals.

Another potential limitation of our investigation was the housing conditions of the animals. The C57BL/6J mouse model was selected due to its generalizability and common use in exercise studies. However, there were issues with in-cage fighting when mice were group housed and therefore some animals had to be individually caged. Social isolation can influence hippocampal plasticity (Stranahan *et al.*, 2006) but it is important to recognize that C57BL/6J male mice can be highly aggressive (Roubertoux *et al.*, 2005) and therefore more research is needed in this mouse strain to understand the influence of individual and group housing. It is not clear which would be more detrimental to hippocampal plasticity, being aggressively attacked or being individually housed. Our mRNA data do not indicate a clear benefit or adverse effect of individual versus group housing in these mice and no individually housed animals were used in the behavioral tasks.

Another factor to consider was that all treadmill running and behavioral testing occurred during the light phase of the light-dark cycle. Mice are nocturnal and perform the majority of their running during the dark cycle. Having mice perform treadmill running and behavioral testing during the light phase may have added an additional level of stress to the exercise that would have been avoided if the tasks were performed during the dark phase. Time of day (Hopkins & Bucci, 2010) and phase of light cycle (Huynh *et al.*, 2011) can influence behavior on anxiety tasks and performing anxiety tasks during the light cycle can even mask the anxiogenic effect of chronic stress (Huynh *et al.*, 2011); however, we observed significant differences in exploratory behavior between exercise and

sedentary mice, suggesting that if the light cycle influenced the data, exercise modulated this effect. The interaction of exercise and light-dark cycle requires further investigation.

Future Directions: Many questions were generated upon examining and interpreting the data from this dissertation project. Even though there is little evidence to suggest that acute exercise induces anxiety in humans, mice are commonly used as a model for exercise research, so understanding the influence of acute exercise on anxiety-like behavior is necessary. Future research should approach acute exercise-induced anxiety with a battery of anxiety tests, potentially in both the light and dark phases. To examine the effects of truly acute bouts of exercise and avoid a training effect, these investigations will require a large sample of animals where each undergoes a session of acute exercise followed by a behavioral task. It remains to be determined if acute exercise will induce anxiety in non-exploratory behavior-dependent tasks and whether motivation or fatigue is a contributing factor to the anxiety-like behavior we observed.

Future research should also attempt to address the fate of the acute exercise-induced *Bdnf IV* mRNA expression. Experiments designed to determine whether *Bdnf* transcript IV is also rapidly translated or is shuttled to dendrites for later translation will be valuable for understanding the importance of the rapid transcription of *Bdnf IV*. In addition, the observations in this dissertation indicate that both acute and chronic exercise increase expression of total *Bdnf*

mRNA to a similar magnitude, suggesting no additional benefit of multiple voluntary wheel running sessions compared to one bout of treadmill exercise. A time course experiment to identify the lasting effects of acute and chronic exercise on *Bdnf* expression is warranted.

The importance of this research lies in its translatability to therapeutic human exercise interventions. The finding in this dissertation that an acute bout of exercise can increase transcription of *Bdnf* even when challenged with compromised LC noradrenergic signaling has important implications. Norepinephrine is important for brain health and plays an important role in defending against Alzheimer's Disease through Bdnf-related signaling mechanisms (Counts & Mufson, 2010; Liu *et al.*, 2015). However, though norepinephrine is important for reducing Alzheimer's Disease pathology, the LC experiences early and rapid neurodegeneration in Alzheimer's Disease (Chalermpalanupap *et al.*, 2013). More research is needed to understand the ability of acute high-intensity exercise to increase *Bdnf* expression after LC lesioning. Use of β-adrenergic blockers such as propranolol will identify if remaining norepinephrine from non-LC sources is influencing this effect. It is possible that overcoming compromised LC function with acute exercise is intensity dependent, which will have important implications for translatability. This is an extremely important area of research and understanding the mechanisms to defend against and treat diseases such as Alzheimer's Disease remains a top priority. This dissertation provides evidence that acute exercise

might be an effective non-invasive therapeutic technique to enhance brain plasticity and may be robust enough to influence brain plasticity even with a compromised noradrenergic system.

Appendix A

mRNA Target	Primer Sequences
Bdnf total	Primer 1: 5' – CCATAAGGACGCGGACTTGTAC -3' Primer 2: 5' – AGACATGTTTGCGGCATCCAGG -3'
Bdnf I	Primer 1: 5'- GACACATTACCTTCCTGCATCT -3' Primer 2: 5'- GGATGGTCATCACTCTTCTCAC -3'
Bdnf II	Primer 1: 5'- GCCTTCATGCAACCGAAGTA -3' Primer 2: 5'- GTGGTGTAAGCCGCAAAGA -3'
Bdnf III	Primer 1: 5'- GCCTTCATGCAACCGAAGTA -3' Primer 2: 5'- GGGCCGGATGCTTCATT -3'
Bdnf IV	Primer 1: 5'- CAGAGCAGCTGCCTTGATGTT -3' Primer 2: 5'- GCCTTGTCCGTGGACGTTTA -3'
Bdnf VI	Primer 1: 5'- GCTGGCTGTCGCACGGTTCCCAGT -3' Primer 2: 5'- GAAGTGTACAAGTCCGCGTCCTTA -3'
ß-AR	Primer 1: 5'- GCCAGATACAATCCATACCATCA -3' Primer 2: 5'- TCGCTATGTTGCTATCACATCG -3'
Pgc-1a	Primer 1: 5' – GGTGTCTGTAGTGGCTTGATTC -3' Primer 2: 5' – GTTCCCGATCACCATATTCCA -3'
tPa (Plat)	Primer 1: 5' – CAACCAAGACCTCCACGA -3' Primer 2: 5' – CACATCCTTCTGCCCACA -3'
ActB	Primer 1: 5'- GAC TCA TCG TAC TCC TGC TTG – 3' Primer 2: 5' – GAT TAC TGC TCT GGC TCC TAG – 3'
NR2A (Grin2a)	Primer 1: 5' – TGC TCA TCA CCT CAT TCT TCT C – 3' Primer 2: 5' – GAT TGA CCT CGC TCT GCT C – 3'
NR2B (Grin2b)	Primer 1: 5' – CAC AAA CAT CAT CAC CCA CAC -3' Primer 2: 5' – TTG ACT TCT CTG TGC CCT TC – 3'
GLUR1 (Gria1)	Primer 1: 5' – TGG CGA GGA TGT AGT GGT A – 3' Primer 2: 5' – AAG AAA AAG GAG AGG CTG GTG – 3'
Gapdh	Primer 1: 5' – AAT GGT GAA GGT CGG TGT G – 3' Primer 2: 5' – GTG GAG TCA TAC TGG AAC ATG TAG – 3'

Appendix B. Neuroreport (2015) vol. 26 (8) pp. 467-472

Title: Lifelong Parental Voluntary Wheel Running Increases Offspring Hippocampal *Pgc-1α* mRNA Expression But Not Mitochondrial Content or *Bdnf* Expression.

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Abstract

When exercise is initiated during pregnancy, offspring of physically active mothers have higher hippocampal expression of brain derived neurotrophic factor (*Bdnf*) and other plasticity and mitochondrial-associated genes, resulting in hippocampal structural and functional adaptations. In the present study, we examined the effects of lifelong parental voluntary wheel running (before, during, and after pregnancy) on offspring hippocampal mRNA expression of genes implicated in the exercise-induced improvement of cognitive function. C57BL/6 mice were individually housed at 8 weeks of age with (EX; n=20) or without (SED; n=20) access to a computer-monitored voluntary running wheel (VRW) for 12 weeks prior to breeding. EX breeders maintained access to the VRW throughout breeding, pregnancy, and lactation. Male offspring were housed in sedentary cages, regardless of parental group, and were sacrificed at 8 (n=18) or 28 weeks (n=19). PCR was used to assess mRNA expression of several genes and mitochondrial content (ratio of mitochondrial to nuclear DNA) in hippocampal homogenates. We found significantly higher peroxisome proliferator-activated receptor γ coactivator 1 alpha (*Pgc-1α)* mRNA expression in EX offspring compared to SED offspring at 8 wks ($p=0.04$), though the effect was no longer present at 28 wks. There was no difference in mitochondrial content or expression of *Bdnf* or any other mRNA targets between offspring at 8 or 28 wks. In contrast to exercise initiated during pregnancy, parental voluntary physical activity initiated early in life and maintained throughout pregnancy has little effect

on offspring mRNA expression of genes implicated in exercise-induced hippocampal plasticity.

Key words

Brain derived neurotrophic factor; BDNF; PGC-1; Exercise; Pregnancy; Offspring; Hippocampus

Introduction

Physical exercise during pregnancy improves health-related outcomes in both mother and offspring [1-3]. In the pregnant mother, exercise can reduce the risk of gestational diabetes, preeclampsia, and excessive gestational weight gain, conditions associated with negative health outcomes in the offspring [2]. Remarkably, exercise during pregnancy also impacts offspring brain health both in early postnatal development and adulthood [4-11]. In humans, *in utero* exercise exposure is associated with greater cognitive performance in early postnatal development [10] and higher intelligence scores at 5 years of age [9] relative to children of mothers that did not exercise during pregnancy. Numerous studies in rodents show that *in utero* exercise exposure increases hippocampal expression of brain derived neurotrophic factor (Bdnf) [4; 5; 7; 12]; cell proliferation and neuron differentiation [5; 6]; increased mitochondrial content and expression of genes associated with mitochondrial biogenesis [13]; and improved performance on spatial [4; 8] and non-spatial memory tasks [5; 7; 14]. In addition to the influence of maternal exercise, long-term paternal forced exercise enhances male offspring neurotrophin expression and spatial learning and memory performance [15], suggesting transgenerational inheritance of exercise effects beyond direct *in utero* exposure. There is remarkable consistency between the effects of *in utero* and adult exercise exposure on the hippocampus. For example, adult exercise exposure increases Bdnf protein and mRNA expression, enhances neurogenesis and cell survival, increases mitochondrial content, and improves learning and memory [reviewed in 16; 17]. Further,

exercise lowers the risk of Alzheimer's disease (AD) in humans and reduces pathology after disease onset in transgenic animals [18]. Similar results are observed following *in utero* exercise exposure in AD transgenic mice [19].

To examine the impact of parental exercise on offspring hippocampal phenotype, researchers have primarily initiated maternal exercise during pregnancy, rather than prior to gestation. Exercise during pregnancy is useful for highlighting the specific effect of *in utero* exercise exposure; however, though beginning exercise during pregnancy is recommended [2], only a low percentage of women report being more active during pregnancy than before [20]. As the physical changes that occur during pregnancy favor a sedentary lifestyle, it is more likely that women who exercise regularly will continue to exercise during pregnancy, while women who are sedentary prior to pregnancy will remain sedentary. Thus, examining the impact of exercise prior to and during pregnancy on offspring phenotypes is important to understand the effectiveness of *in utero* exercise exposure for enhancing brain health. For this reason we investigated the influence of lifelong parental exercise on offspring hippocampal gene expression and mitochondrial copy number at two different offspring ages. Our gene targets were specifically selected based on previous literature reporting sensitivity to adult exercise training and/or *in utero* exercise exposure and we hypothesized that mRNA for *Bdnf* (and related processing and signaling markers), growth factors, the mitochondrial biogenesis regulator peroxisome proliferator-activated receptor γ coactivator 1 α (*Pgc-1α*), and synaptic markers would be elevated in offspring of lifelong physically active parents.

Methods

Animals and Experimental Design

All animal procedures were performed in accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the University of Maryland. This investigation was part of a larger investigation designed to examine whole body and tissue (skeletal muscle, white adipose, liver) metabolic phenotypes in multiple generations of offspring of exercised vs. sedentary parents. The availability of these mice offered a unique opportunity to test an equally important yet unrelated hypothesis that lifelong parental physical activity increases plasticity associated mRNA expression in offspring hippocampus.

Twenty male and twenty virgin female eight-week-old C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA) were randomly separated into individual cages with (F_0 EX) or without (F_0 SED) access to a computer monitored running wheel (Lafayette Instruments, Lafayette IN). Though individual housing may influence behavior and hippocampal plasticity it does not influence rodent running behavior [21] and is consistent with previous investigations of maternal exercise [4-8]. After 12 weeks of voluntary wheel running, males were randomly housed with females from like groups (1 male and 1 female per cage; EX with EX and SED with SED). During the breeding period, both males and females in the EX mating group maintained running wheel access; however, running activity could not be monitored during this period.

Males were removed after pregnancy was visually confirmed by vaginal plug or after 2 weeks of pairing. EX females maintained running wheel access during pregnancy and lactation. Two F_0 EX breeding pairs did not produce viable F_1 offspring. The resulting offspring made up the F_1 generation. Average litter size for F_1 offspring was 6.1 ± 0.6 EX and 6.4 ± 0.5 SED offspring/litter; there was no significant difference in litter size between groups. Litters with 8 or fewer offspring were included for analysis. Only male offspring are presented due to fewer female mice available from exercised parents compared to sedentary parents. F_1 males were weaned at 21 days of age, group-housed in standard cages without running wheel access and were sacrificed at 8 (n=18) or 28 weeks (n=19). The animals sacrificed at 28 weeks were bred at 8 weeks of age and individually housed thereafter until sacrifice. No more than 3 offspring per litter were studied per age group. A standard diet (Purina Mills LLC, St. Louis, MO, USA; RMH 3000; 60% carbohydrate, 14% fat, and 26% protein) and water were provided *ad libitum* to animals of all experimental groups.

Tissue Collection & Processing

Twenty-four hours prior to sacrifice, all F_1 mice were exposed to intraperitoneal glucose tolerance testing. This procedure was performed to address the overall hypothesis of the investigation, though the data will not be discussed in this report. Euthanasia by exsanguination by cardiac puncture followed by removal of the heart was performed under isoflurane anesthesia. The hippocampus was isolated, halved, and immediately frozen in liquid nitrogen. Prior to nucleic acid

isolation, hippocampi were homogenized using a glass Dounce homogenizer. Total RNA was isolated with TRIzol reagent (Life Technologies, Grand Island, NY, USA) following manufacturers instructions and quantified via spectrophotometry. Reverse transcription was performed with 1 µg of total RNA with the High-Capacity cDNA RT kit (Life Technologies). Following RNA isolation DNA was isolated from TRIzol reagent and quantified via spectrophotometry.

Gene Expression

Real-time quantitative PCR was used to assess mRNA expression of total brain derived neurotrophic factor (*Bdnf*; exon IX), *Bdnf* exon IV (*Bdnf IV*), *Pgc-1α*, tissue plasminogen activator (*tPa*), and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*; expression control). Primer and probe sequences were purchased pre-made *(Pgc-1α, tPa, Gapdh)* or designed (*Bdnf IX, Bdnf IV*) for the mRNA sequence of each gene using Integrated DNA Technologies' PrimeTime qPCR Assay designer. Primer sequences are listed in Supplemental Table 1. *Bdnf IV, Pgc-1α, tPa*, and *Gapdh* primer pairs spanned exons to prevent amplification of genomic DNA. Because *Bdnf* total is represented by amplification of exon IX, the protein coding exon that is present in each transcript, this primer pair could not span exons. Efficiency for each primer:probe assay was determined prior to use. RT-PCR was used to measure the expression of insulin like growth factor 1 (*Igf1*), vascular endothelial growth factor (*Vegf*), neurotrophic tyrosine kinase receptor type 2 (*TrkB*), calpactin (*p11*), synapsin 1 (*Syn1*), synaptobrevin (*Vamp2*), synaptotagmin 1 (*Syt1*), and synaptophysin (*Syp*).

Gapdh was used as an expression control for RT-PCR. All RT-PCR primers were designed to span exons.

Mitochondrial Copy Number

DNA was subjected to real-time quantitative PCR and comparison of *β-actin* and *cytochrome b* amplification was used to determine the relative amounts of nuclear and mtDNA, respectively. These primer:probe assays were purchased pre-made from Integrated DNA Technologies and efficiency tested prior to use. Primer sequences are listed in Supplemental Table 1.

Statistical Analysis

Unpaired t-tests were used to compare gene expression between EX and SED groups within age using SAS version 4.2. One-tailed t-tests were used to examine *Bdnf* mRNA expression, *Pgc-1α* mRNA expression, and mitochondrial copy number. Two-tailed t-tests were used to examine all other mRNA targets. Significance was achieved at $p<0.05$.

Results

F_0 Wheel Activity

Running data for the F_0 breeders are shown in Figure 1. Peak running was achieved during week 4 (6689 meters/24hrs) for males and week 2 for females (7209 meters/24hrs). In males there was a steady decline in running activity until the final week, when the lowest running distances were recorded (1505

meters/24hrs). In females, lowest activity was recorded during pregnancy (347 meters/24hrs). Running activity increased to pre-pregnancy levels following pregnancy.

F_1 Eight-Week Offspring Outcomes

Hippocampal gene expression data for F_1 8-week offspring are shown in Figure 2. Eight-week old offspring of EX parents had significantly higher *Pgc-1α* mRNA expression compared to offspring of SED parents ($p=0.04$, Fig. 2a). We observed no significant differences between offspring of EX and SED parents in any other targets measured (Fig. 2b). There was no effect of parental exercise on offspring *Gapdh* expression (confirmed with both qPCR and gel-based RT-PCR). We also observed no differences between offspring of EX and SED parents in mitochondrial copy number (Figure 3).

F_1 Twenty-Eight Week Offspring Outcomes

Hippocampal gene expression data for F_1 28 week are shown in Figure 4. The difference in *Pgc-1α* mRNA expression observed in 8-week old animals was no longer present in the 28-week old offspring. We observed no differences any mRNA target between 28-week old offspring of EX and SED parents. There was no effect of parental exercise on offspring *Gapdh* expression (confirmed with both qPCR and gel-based RT-PCR). We observed no differences in mitochondrial copy number between 28-week-old offspring of EX and SED parents (Fig. 3).

Discussion

We report here that parental exercise training prior to, during, and after (lactation) gestation results in greater hippocampal *Pgc-1α* gene expression at 8 weeks of age in male EX offspring that returns to baseline by 28 weeks of age. This change in *Pgc-1α* expression was not accompanied by higher mitochondrial copy number, as might be expected based on the known role of *Pgc-1α* in mitochondrial biogenesis [22; 23]. In contrast to previous studies initiating exercise during pregnancy, we observed no significant difference in *Bdnf* mRNA expression between offspring of EX and SED parents at 8 or 28 weeks of age.

Maternal exercise beginning during pregnancy has numerous health benefits to offspring. In the offspring hippocampus, maternal exercise (whether swimming, treadmill running, or voluntary wheel running) beginning during pregnancy leads to changes in Bdnf mRNA and protein expression [4; 5; 7; 12], enhanced learning and memory performance [4; 5; 7; 8; 14], neurogenesis [5; 6], mitochondrial biogenesis, and mRNA expression of genes associated with mitochondrial biogenesis and oxidative metabolism [13]. We observed that maternal and paternal exercise beginning early in life and continuing through mating, gestation, and lactation resulted in no difference in male offspring hippocampal mRNA expression of any of our targets with the exception of *Pgc-1α*. *Pgc-1α* was elevated at 8 weeks in offspring of EX parents, though returned to baseline by 28 weeks. *Pgc-1α* is a co-transcription factor that is considered a regulator of mitochondrial biogenesis. When co-expressed with other tissue- and

temporal-specific transcription factors, *Pgc-1α* stimulates the transcription of genes necessary for mitochondrial biogenesis [24]. *Pgc-1α* expression is induced in many tissues, including the brain, in response to physical exertion [17; 23]. Using three different exercise durations, Park et al. [13] showed that offspring of treadmill-exercised mothers had more hippocampal mitochondria, determined by measuring mitochondrial DNA relative to nuclear DNA, and greater levels of Pgc-1α protein expression. This effect was observed in offspring of mothers who exercised the longest of three exercise durations. Even though we observed a significant effect of parental exercise on offspring *Pgc-1α* expression at 8 weeks, we did not observe an accompanying increase in mitochondrial content at 8 or 28 weeks. Potentially, we missed the window of observation for a change in mitochondrial content that took place between 8 and 28 weeks before returning to baseline.

Based on previous literature, we hypothesized that parental physical activity prior to, during, and after gestation would result in an increase in neurotrophin and growth factor mRNA expression, however this hypothesis was not supported. A number of studies have reported an effect of maternal and paternal exercise on offspring hippocampal *Bdnf* expression, though how long this effect persists still remains to be determined. Parnpiansil et al. [4] showed that treadmill exercise in pregnant rats resulted in greater offspring hippocampal *Bdnf* mRNA expression at post-natal (PN) day 0 (PN0), no difference at PN14, and significantly lower *Bdnf* expression at PN28. In contrast, Kim et al. [7] and Lee et al. [5] showed that *Bdnf* mRNA expression was elevated in offspring of

treadmill- and swim-trained mothers, respectively, at 29 days after birth. Even though these studies reported significantly greater *Bdnf* expression in offspring of exercise mothers compared to offspring of sedentary mothers one month after birth, our youngest time point was two months of age which may explain why even in our youngest group of offspring, we observed no differences in neurotrophin (*Bdnf*) or growth factor (*Igf1* and *Vegf*) mRNA expression. Yin et al. [15] reported enhanced *Bdnf* expression (mRNA and protein) and spatial memory performance in ~one-month old male offspring of treadmill exercised male C57BL/6J mice. Again, this is much younger than our youngest group, suggesting the influence of parental exercise is relatively short-lived. It is important to note that the offspring in these studies, and in our study, were all sedentary and it is possible that offspring of physically active parents would maintain the benefits of parental physical activity if they themselves are provided access to exercise.

Though we did not observe differences in synaptic (*Syn1*, *Vamp2*, *Syt1*, *Syp*), neurotrophin (*Bdnf, Bdnf IV),* neurotrophin receptor (*TrkB),* posttranslational processor (*tPa, p11*), or growth factor (*Vegf, Igf1*) mRNA expression, structural adaptations may have occurred that were not represented by differences in mRNA expression. Bick-Sander et al [6] reported differences between offspring of exercise (beginning during pregnancy) and sedentary mothers in neuron survival and total granule cell numbers at PN36, despite no significant differences in mRNA expression of *Vegf, Fgf-2, or Igf1*. These data suggest lasting structural changes that are not mirrored by lasting differences in

mRNA expression. Future studies are needed to determine if lifelong parental physical activity influences hippocampal structure and function in offspring in the absence of differences in mRNA expression.

In addition to the age at which the offspring were examined, another difference between our study and previous studies is the timing and mode of exercise exposure. We provided mice with a running wheel for 12 weeks prior to mating and pregnancy. Previous studies have used forced exercise modes such as swimming or treadmill running, which add additional stress, but also have the benefit of controlled exercise duration (swimming and treadmill) and intensity (treadmill). Studies that have used voluntary wheel running have initiated exercise during pregnancy. In addition to the influence of novelty/enrichment, mice are very active in the first few weeks of wheel exposure, with declining activity thereafter (Fig 1). Compared to Bick-Sander et al. [6], who used the same strain of mice as our study, our pregnant mice ran much less. Between days 5 and 10 of pregnancy, mice in the Bick-Sander study ran between 2500 and 3000 meters/day, while in our study, pregnant mice ran less than 700 meters/day (see Fig 1). The dependence of the exercise volume is also supported by investigations using forced exercise. Park et al. [13] reported that offspring of the most active pregnant mice were significantly different than controls when hippocampal mitochondrial content, mitochondrial enzyme activity, and Pgc-1α protein expression were assessed.

A limitation of this investigation was the exposure of offspring to glucose tolerance testing, which may have added additional stress to the animals and influenced hippocampal gene expression. Weekly handling of our mice to prepare them for the stress of the GTT likely reduced the stress response, though we cannot rule out an influence of the procedure on gene expression. Another limitation of our study is that we did not observe and record maternal care, which can strongly influence hippocampal development and plasticity [25]. Though not a limitation of our study, it is possible that providing offspring access to a voluntary running wheel may expose an exercise-induced adaptation to parental exercise, which provides these mice with a plasticity advantage above offspring of sedentary parents. This is a question that should be addressed in future investigations.

We believe that our data, in the light of previously reported findings, suggest a high volume and/or intensity of physical activity during pregnancy must be maintained to observe the beneficial effects of maternal physical activity on hippocampal plasticity-associated gene expression and mitochondrial copy number. The steady decrease in voluntary wheel running observed over the 12 weeks prior to mating, in both parents, and during pregnancy in mothers, may have prevented the strong effect observed in other investigations. Treadmill exercise may be more effective at maintaining intensity and volume prior to and during pregnancy.

Competing interest

None declared

Funding

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Authors' contributions.

ACV, LMG, EES, and SMR designed the study; ACV and LMG collected the data; data analysis, preparation of figures, and drafting the manuscript was done by ACV; ACV, LMG, EES, and SMR edited and revised this manuscript; and all authors approved the final version.

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Figure 1. Average running activity of mice. Data are shown as average distance run over 24 hours per week. Running data were not collected during mating due to the presence of two mice in the cage and the inability to determine which mouse was using the running wheel. The litters were delivered between 23 and 24 weeks on the timeline. There was a six-day span between first and last litter, thus pre-weaned mice had access to the wheel. We cannot rule out the possibility that pre-weaned mice are contributing to recorded wheel revolutions during week 27.

Figure 2 (A-B). Whole hippocampal homogenate mRNA levels in F_1 8 week old male offspring of exercise (n=10) and sedentary (n=8) parents. Bars represent mean (±SEM) mRNA expression relative to *Gapdh* mRNA expression. * denotes significance at $p<0.05$.

Figure 3. Mitochondrial DNA content in F_1 8 wk and 28wk old male offspring of exercise and sedentary parents. There were no significant differences in *CytB* mitochondrial DNA content relative to *ActB* DNA content between offspring of exercise and sedentary parents at 8 weeks or 28 weeks of age. Results are means ±SEM.

Figure 4 (A-B). Whole hippocampal homogenate mRNA levels in F_1 28 week old male offspring of exercise (n=9) and sedentary (n=10) parents. Bars represent mean (±SEM) mRNA expression relative to *Gapdh* mRNA expression. * denotes significance at $p<0.05$.

Supplemental Table 1. Primer sequences for genes of interest.

Figure 1.

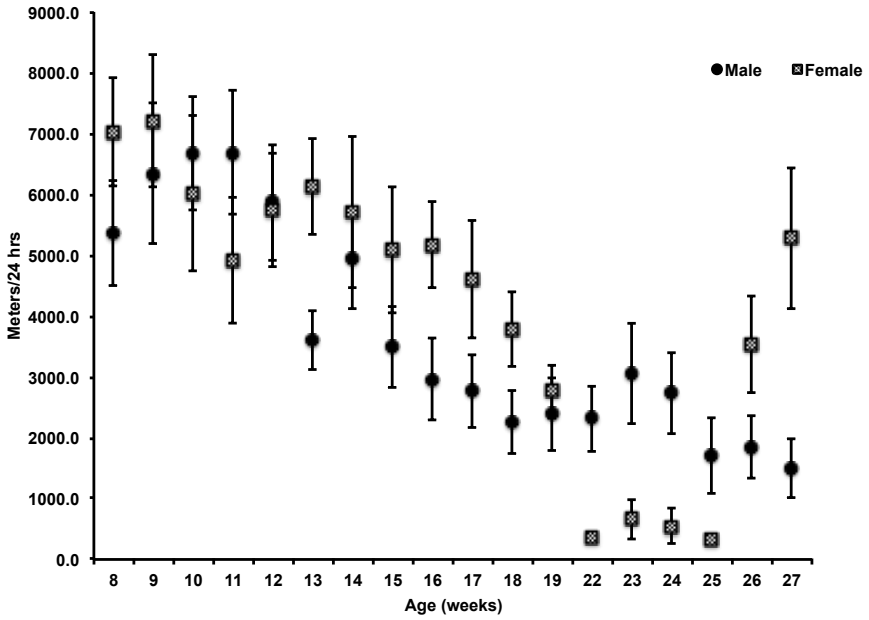

9000.0
8000.0
7000.0
6000.0
5000.0
4000.0
3000.0
2000.0
1000.0
0.0
Meters/24 hrs
Male
Female
8
9
10
11
12
13
14
15
16
17
18
19
22
23
24
25
26
27
Age (weeks)

Figure 2.

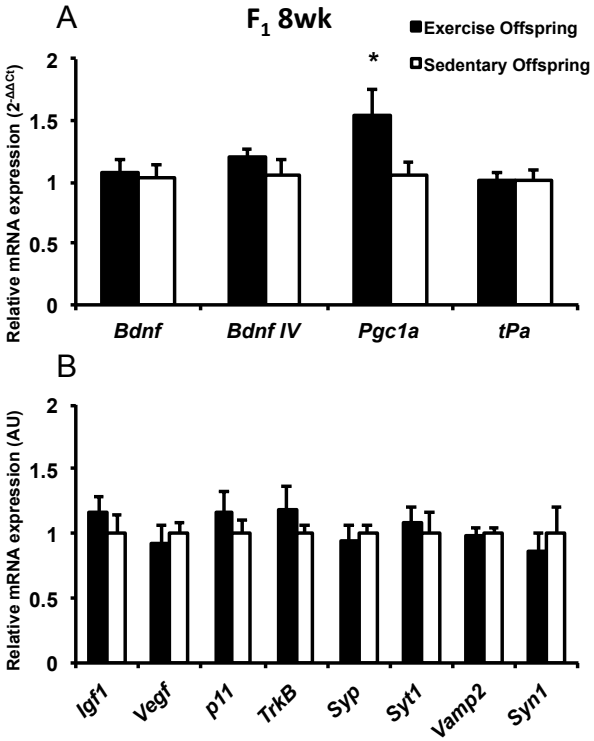

A
F1 8wk
Exercise Offspring
Sedentary Offspring
*
Relative mRNA expression (2-ΔΔCt)
2
1.5
1
0.5
0
Bdnf
Bdnf IV
Pgc1a
tPa
B
Relative mRNA expression (AU)
2
1.5
1
0.5
0
Igf1
Vegf
p11
TrkB
Syp
Syt1
Vamp2
Syn1

Figure 3.

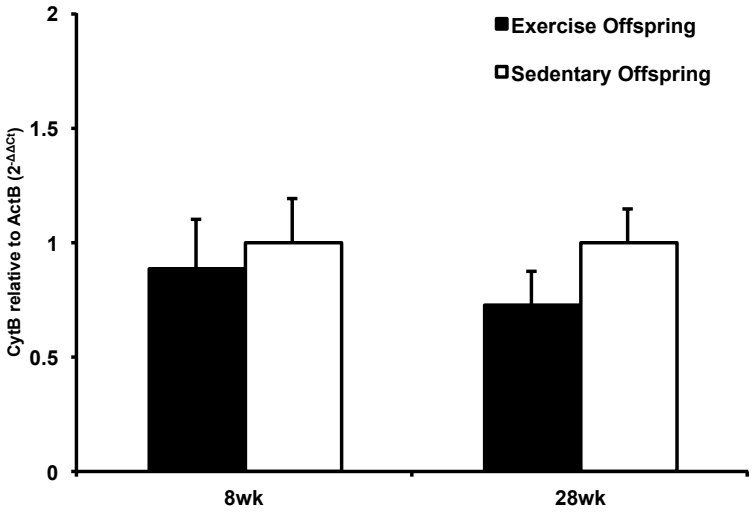
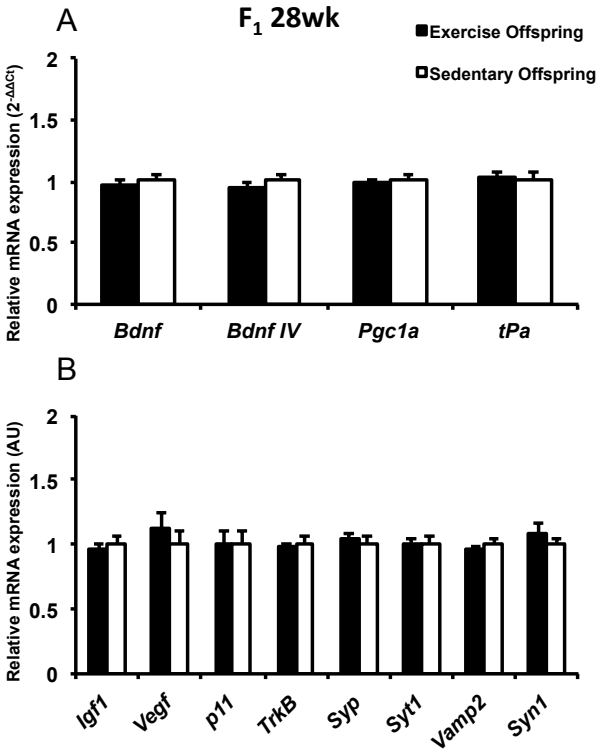

Exercise Offspring
Sedentary Offspring
CytB relative to ActB (2-ΔΔCt)
2
1.5
1
0.5
0
8wk
28wk

Figure 4.


A
F1 28wk
Exercise Offspring
Sedentary Offspring
Relative mRNA expression (2-ΔΔCt)
2
1.5
1
0.5
0
Bdnf
Bdnf IV
Pgc1a
tPa
B
Relative mRNA expression (AU)
2
1.5
1
0.5
0
Igf1
Vegf
p11
TrkB
Syp
Syt1
Vamp2
Syn1

Supplemental Table 1.

mRNA Target	Primer Sequences
Bdnf total	Primer 1: 5' – CCATAAGGACGCGGACTTGTAC -3' Primer 2: 5' – AGACATGTTTGCGGCATCCAGG -3'
Bdnf IV	Primer 1: 5'- CAGAGCAGCTGCCTTGATGTT -3' Primer 2: 5'- GCCTTGTCCGTGGACGTTTA -3'
Pgc-1a	Primer 1: 5' – GGTGTCTGTAGTGGCTTGATTC -3' Primer 2: 5' – GTTCCCGATCACCATATTCCA -3'
tPa (Plat)	Primer 1: 5' – CAACCAAGACCTCCACGA -3' Primer 2: 5' – CACATCCTTCTGCCCACA -3'
Syt1	Forward: 5'- CGG TCC TCG CTC CAG TTT CCC – 3' Reverse: 5' – CGG GAC GGC AAG GGC AAT GT – 3'
Syp	Forward: 5' – GCC GAC TGG GCT GTT CCG AC – 3' Reverse: 5' – CCC CCA GCC ACC AGC TGA TTC – 3'
Vamp2	Forward: 5' – CAG GCC CAG GTG GAT GAG GTG GT -3' Reverse: 5' – CTG GAG GGC ATC TGC ACG GTC – 3'
TrkB	Forward: 5' – GCA TGA AAG GCC CAG CTT CGG T – 3' Reverse: 5' – GGG ACC GCC CTC CGA AGA AGA – 3'
Vegf	Forward: 5' – GCC GAG CTC ATG GAC GGG TG – 3' Reverse: 5' – GGT GCA GCC TGG GAC CAC TTG – 3'
Gapdh	Forward: 5' – TCA AGC TCA TTT CCT GGT ATG ACA – 3' Reverse: 5' – TCT TGC TCA GTG TCC TTG CT -3'
Syn1	Forward: 5' – GCC AAT GGT GGA TTC TCT GT -3' Reverse: 5' – CAG CAC AAA GTC TGG CTT CA -3'
Igf1	Forward: 5' – TGG ATG CTC TTC AGT TCG TG – 3' Reverse: 5' – GCA ACA CTC ATC CAC AAT GC – 3'
p11	Forward: 5' – GCG TAC AAA GAC CGC CGG TC - 3' Reverse: 5' – GTC GAA ACC TGG GCC CCG AAG -3'
β-actin	Primer 1: 5' – CTTGATCTTCATGGTGCTAGGAG -3' Primer 2: 5' – CGTTGACATCCGTAAAGACCT -3'
Cytochrome B	Primer 1: 5' – TATTCCTTCATGTCGGACGA -3' Primer 2: 5' – AAATGCTGTGGCTATGACTG -3'

Appendix C

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Education

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Ph.D. Neuroscience and Cognitive Science
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January 2008 to May 2009
M.S. Exercise Science
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Publications

Refereed Research Articles:

1. **Venezia, A.C.,** Guth, L.M., Sapp R. M., Spangenburg, E.E., Roth, S.M. Sex-dependent and independent effects of long-term voluntary wheel running on hippocampal Bdnf expression. Submitted to *Physiology and Behavior.*

2. **Venezia A.C**., Guth L.M., Spangenburg E.E., Roth S.M. (2015). Lifelong Parental Voluntary Wheel Running Increases Offspring Hippocampal *Pgc-1α* mRNA Expression But Not Mitochondrial Content or *Bdnf* Expression. *Neuroreport* 26 (8): 467-72.

3. Guth L.M., Ludlow A.T., Witkowski S., Lima L., Marshall M.R., **Venezia A.C**., Xiao T., Lee M.L., Spangenburg E.E., Roth S.M. (2013). Sex-Specific Effects of Exercise Ancestry on Metabolic, Morphological, and Gene Expression Phenotypes in Multiple Generations of Mouse Offspring. *Exp Physiol* 98 (10): 1469–1484.

Invited Reviews:

1. **Venezia A.C**., Roth S.M. (2015). Recent research in the genetics of exercise training adaptation *Medicine and Sport Science - 'Genetics and Sports', 2nd revised and extended edition.* In Press.

2. Rawson E.S., **Venezia A.C.** (2011). Use of Creatine in the Elderly and Evidence for Effects on Cognitive Function in Young and Old. *Amino Acids* 40(5):1349-62.

In Preparation:

1. **Venezia, A.C.,** Quinlan, E.M., Roth S.M. The Influence of Acute and Chronic Exercise on Hippocampal GluR1 and *Bdnf* Transcript Expression. In preparation for *Genes, Brain and Behavior.*

2. **Venezia, A.C.,** Hyer, M.M., Glasper E.R., Roth S.M., Quinlan, E.M. The Effect of One Bout of Acute Exercise on AMPA Receptor Phosphorylation, *Bdnf* Expression, and Behavior. In preparation for *Genes, Brain and Behavior.*

3. Guth L.M., **Venezia A.C.,** Marini M.P., Beltran, E.P., Spangenburg, E.E., Roth S.M. Effects of Exercise Ancestry on Metabolic, Morphological, and Gene Expression Phenotypes in Multiple Generations of Mature Mouse Offspring. In preparation for *Experimental Physiology*.

Presentations

Invited Presentations:

1. **Venezia, A.C.** Acute Exercise May Induce Hippocampal Plasticity…Or Anxiety. NACS Research Day, University of Maryland

2. Panel Discussion with: Clevenger S., **Venezia, A.C**., Patel P. Science, Social Responsibility, and Crisis. 8th Annual Physical Cultural Studies Graduate Student Conference: "Bodies, Science and Technology," University of Maryland

Research Presentations:

1. Steele, C.N., Fradkin, A.J., Andreacci, J.L., **Venezia, A.C.,** Rawson, E.S. Effects of Age and Sex on Muscle Function During Isovelocity Contractions. Slide presentation. *Annual Meeting of the Mid-Atlantic Chapter of the American College of Sports Medicine*, **October 2014**, Harrisburg, PA.

2. **Venezia, A.C.,** Guth, L.M., Spangenburg, E.E., Roth, S.M. Sex-dependent and independent effects of long-term voluntary wheel running on hippocampal gene expression. Poster presentation, *American College of Sports Medicine Annual Meeting.* **June 2014,** Orlando, FL.

3. Kayes, M. K., **Venezia, A.C**., Sprenger, A.M., Roth, S.M., Dougherty, M.R., Bolger, D.J., Hatfield, B.D., Variability in Learning in Adults Explained by Cardiovascular Fitness, Physical Activity, and APOE Genotype. Poster presentation, *American College of Sports Medicine Annual Meeting.* **June 2014,** Orlando, FL.

4. **Venezia, A.C.,** Guth, L.M., Marini, M.P., Smith, J.C., Spangenburg, E.E., Roth, S.M. Impact of Parental Voluntary Wheel Running on Offspring Hippocampal Gene Expression in C57BL/6 Mice. Poster presentation, *Neuroscience 2012*, **October 2012**, New Orleans, LA.

5. **Venezia, A.C.,** Guth, L.M., Marini, M.P., Beltran, E.P., Spangenburg, E.E., Roth, S.M. Effects of Parental Physical Activity on Hippocampal Gene Expression in C57BL/6 Mice. Poster presentation, *American College of Sports Medicine Annual Meeting.* **June 2012,** San Francisco, CA.

6. Guth, L.M., **Venezia, A.C.,** Marini, M.P., Beltran, E.P., Spangenburg, E.E., Roth, S.M. Effects of Physical Activity Ancestry on Aspects of Body Composition and Glucose Tolerance in Mice. Poster presentation, *American College of Sports Medicine Annual Meeting.* **June 2012,** San Francisco, CA.

7. Marini, M.P., Guth, L.M., **Venezia, A.C.,** Beltran, E.P., Spangenburg, E.E., Roth, S.M. Effects of Chronic Exercise on DNA Methyltransferase Expression in Mouse Testes. Poster presentation, *American College of Sports Medicine Annual Meeting*. **June 2012,** San Francisco, CA.

8. Rawson, E.S., **Venezia, A.C.,** Still, C.D. No Adverse Effects Associated with Low-Dose Longer-Duration Creatine Supplementation in Older Adults. Poster presentation, *American College of Sports Medicine Annual Meeting.* **May 2012,** San Francisco, CA.

9. Guth L.M., Ludlow A.T., Witkowski S., Marshall M.R., Lima L., **Venezia A.C.**, Xiao T, Lee M-L.T., Spangenburg E.E., and Roth S.M. Exercise ancestry decreases lipogenesis-related gene expression in skeletal muscle of male offspring. Oral and poster presentation, *Experimental Biology 2011*, **March 2011**, Washington, D.C. *FASEB J* 25:862.3.

10. **Venezia, A.C**., Ludlow, A.T., Witkowski, S., Marshall, M.R., Spangenburg, E.E., Roth, S.M., Effect of one year of voluntary wheel running on transcript specific hippocampus *Bdnf* gene expression. Poster presentation, *ACSM's Conference on Integrative Physiology of Exercise*, **September 2010,** Miami Beach, FL.

11. Guth L.M., Ludlow A.T., Witkowski S., Marshall M.R., Lima L., Perret K., Caffes N., **Venezia A.C**., Spangenburg E.E., and Roth S.M. Transgenerational effects of physical activity ancestry on mouse body composition, glucose metabolism, and gene expression. Poster presentation, *ACSM's Conference on Integrative Physiology of Exercise*, **September 2010**, Miami Beach, FL.

12. **Venezia, A.C**., Ludlow, A.T., Witkowski, S., Marshall, M.R., Spangenburg, E.E., Roth, S.M., Effect of one year of voluntary wheel running on transcript specific hippocampus *Bdnf* gene expression. Slide Presentation, Department of Kinesiology Annual Exercise Physiology Retreat, **August 2010,** Beltsville, MD

13. **Venezia, A.C**., Smoliga, J., Still, C.D., Rawson, E.S. Are Older Adults Less Physically Active than Young Adults? Presented updated data as a free communication slide presentation at the *Annual Meeting of the Mid-Atlantic Chapter of the American College of Sports Medicine*, **November 2009**, Harrisburg, PA.

14. **Venezia, A.C.,** Wierzbicki, J.S., Reitmeyer, D., Shick, A., Still C.D., Rawson, E.S. Physical Activity and Cognitive Processing in Older Men and Women. Poster presentation, *American College of Sports Medicine National Conference*, **May 2009**, Seattle, WA.

15. **Venezia, A.C.,** Wierzbicki J. S., Reitmeyer D, Schick A, Rawson E.S., Physical Activity and Cognitive Processing in Older Men and Women. Free communication slide presentation, *Annual Meeting of the Mid-Atlantic*

Chapter of the American College of Sports Medicine, **November 2008**, Harrisburg, PA.

16. Wierzbicki, J.S., Reitmeyer, D., Shick, A., **Venezia, A.C**., Still, C.D., Rawson, E.S. Are Older Adults Less Physically Active than Young Adults? **Presented for Jackie Wierzbicki** as a free communication slide presentation, *Annual Meeting of the Mid-Atlantic Chapter of the American College of Sports Medicine*, **November 2008**, Harrisburg, PA.

Chaired Sessions:

1. **Venezia, A.C.** (Chair) Genetics of Physical Activity, Exercise Training, and Sport Performance. MARC-ACSM Annual Meeting, Harrisburg, PA, 2015

2. **Venezia, A.C.** (Chair) Mid-Atlantic Regional Chapter of American College of Sports Medicine Student College Bowl. MARC-ACSM Annual Meeting, Harrisburg, PA, 2014

3. **Venezia, A.C.** (Chair) Meet the Experts. MARC-ACSM Annual Meeting, Harrisburg, PA, 2014

4. **Venezia, A.C.** (Chair) Mid-Atlantic Regional Chapter of American College of Sports Medicine Student College Bowl. MARC-ACSM Annual Meeting, Harrisburg, PA, 2013

5. **Venezia, A.C.** (Chair) Meet the Experts. MARC-ACSM Annual Meeting, Harrisburg, PA, 2013

Research Support

August 2014 – January 2016
National Institute of Mental Health of the National Institutes of Health, National Research Service Award. Individual Pre-Doctoral Fellowship ($56,306). Title: Acute Exercise and Hippocampal Plasticity.

June 2014 – 2015
University of Maryland, College Park Department of Kinesiology. Graduate Research Initiative Program Grant ($2,500). Title: Acute Exercise, Catecholamines, and Hippocampal Plasticity.

December 2012 – 2013
University of Maryland, College Park Department of Kinesiology. Graduate Research Initiative Program Grant ($2,500). Title: Acute Exercise and AMPA Receptor GluR1 Subunit Phosphorylation.

February 2010 – 2011
University of Maryland, College Park Department of Kinesiology Graduate Research Initiative Program Grant ($2,500). Title: Effect of One Year of Voluntary Wheel Running on DNA Methylation in the Hippocampus.

Awards and Honors

Spring 2009
Bloomsburg University Bill Sproule Award: Senior Exercise Science Major with the highest
GPA.

Teaching Experience

Instructor:

University of Maryland, College Park

Spring 2016
SPHL498F Social, Political, & Ethical Issues in Public Health Instructor

University of Maryland, Shady Grove

Fall 2015
- KNES360 Physiology of Exercise (Co-Instructor)

Teaching Assistant:

University of Maryland College Park

Semester	Course
Fall 2009	KNES360 - Physiology of Exercise Laboratory Instructor (TA) KNES360 - Physiology of Exercise Laboratory Instructor (TA) KNES132 - Beginning Badminton KNES160 - Beginning Volleyball
Spring 2010	KNES360 - Physiology of Exercise Laboratory Instructor (Lead TA) KNES360 - Physiology of Exercise Laboratory Instructor (Lead TA) KNES100 - Intermediate Basketball
Summer 2010	KNES157 - Beginning Weight Training KNES157 - Intermediate Weight Training

Fall 2010	KNES360 - Physiology of Exercise Laboratory Instructor (Lead TA) KNES360 - Physiology of Exercise Laboratory Instructor (Lead TA) KNES100 - Intermediate Basketball
Fall 2011	KNES360 – Physiology of Exercise Laboratory Instructor (Lead TA) KNES360 – Physiology of Exercise Laboratory Instructor (Lead TA) *Following the fall 2011 semester I restructured the weekly laboratory ass future semesters. These changes remain the format for the course.*

Academic Research Positions

University of Maryland

January 2012 to Present

- National Institute of Health Pre-doctoral Research Trainee

January 2011 to August 2011

- Research assistant: Dr. Stephen Roth's NIH Funded "Role of Maternal Exercise Environment on Transgenerational Offspring Health."

Bloomsburg University

January 2008 to July 2009

- Research assistant: Dr. Eric Rawson's NIH Funded "Creatine Supplementation and Cognitive Function in the Elderly."

Job Experience

February 2009 to May 2009
Intern, Geisinger Core Genomics Laboratory, Danville, PA.

September 2006 to June 2009
Fitness Aide, Geisinger Fitness Center, Danville, PA.

June 2007 to July 2007
Intern, Geisinger Medical Center Stress Laboratory and Cardiac Rehabilitation Program, Danville, PA.

June 2006 to August 2006
Trainer, Edge Fitness and Martial Arts, Towanda, PA.

Professional Memberships

American College of Sports Medicine

Mentoring

August 2013 to July 2014
Matthew Ballew – Senior Kinesiology/Pre-medicine Student

August 2012 to May 2013
Rhea Ramakrishnan – Sophomore Biology Student

August 2011 to May 2012
Kelly Protzko – Senior Kinesiology Honors Student. Mentored through senior thesis project.

Service

University of Maryland

Department of Kinesiology:

October 2014 to May 2015
Student Representative on Kinesiology Department Graduate Committee

August 2012 to August 2013
Kinesiology Department Graduate Student Council

School of Public Health:

August 2013 to August 2014
School of Public Health Senate Executive Committee (elected position)

Neuroscience and Cognitive Science Program:

August 2013 to present
Neuroscience and Cognitive Science Recruitment Fest Committee

Bloomsburg University

September 2008 to May 2009
Bloomsburg University Student Grievance Board

American College of Sports Medicine

May 2014 to Present

American College of Sports Medicine Student Affairs Committee

May 2013 to June 2015
Student Representative on Mid-Atlantic Regional Chapter of American College of Sports Medicine Executive Board

Bibliography:

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Akhavan, M.M., Emami-Abarghoie, M., Safari, M., Sadighi-Moghaddam, B., Vafaei, A.A., Bandegi, A.R., & Rashidy-Pour, A. (2008) Serotonergic and noradrenergic lesions suppress the enhancing effect of maternal exercise during pregnancy on learning and memory in rat pups. *Neuroscience*, **151**, 1173–1183.

Allgrove, J.E., Gomes, E., Hough, J., & Gleeson, M. (2008) Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. *Journal of Sports Sciences*, **26**, 653–661.

Alomari, M.A., Khabour, O.F., Alzoubi, K.H., & Alzubi, M.A. (2013) Forced and voluntary exercises equally improve spatial learning and memory and hippocampal BDNF levels. *Behavioural Brain Research*, **247**, 34–39.

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triggered events in the rat hippocampus are required for both short- and long-term memory formation. *Hippocampus*, **12**, 551–560.

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Babaei, P., Damirchi, A., Mehdipoor, M., & Tehrani, B.S. (2014) Long term habitual exercise is associated with lower resting level of serum BDNF. *Neuroscience Letters*, **566**, 304–308.

Baj, G., D'Alessandro, V., Musazzi, L., & Mallei, A. (2012) Physical exercise and antidepressants enhance BDNF targeting in hippocampal CA3 dendrites: further evidence of a spatial code for BDNF splice variants.

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chronic treadmill and wheel running on behavior in rats. *Brain Research*, **1019**, 84–96.

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